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(54) Title: CHIMERIC CHEMOKINE-ANTIGEN POLYPEPTIDES AND USES THEREFOR

(57) Abstract: The present invention provides chimeric polypeptides comprising: (a) one or more chemokine polypeptides selected from the group consisting of (i) chemokines and (ii) polypeptides within one or more of the following groups: fragments of chemokines, analogues of chemokines, derivatives of chemokines, and truncation isoforms of chemokines; (b) one or more antigenic polypeptides; and (c) one ore more polypeptide linkers connecting the one ore more chemokine polypeptides to the one or more antigenic polypeptide(s), and methods of making such chimeric polypeptides and methods of using such polypeptides for eliciting or enhancing an immune response.

CHIMERIC CHEMOKINE-ANTIGEN POLYPEPTIDES AND USES THEREFOR

1. Introduction

The present invention relates generally to chimeric chemokine-antigen polypeptides and methods for using such polypeptides for enhancing an immune response.

2. Background of the Invention

2.1 HIV Vaccines

Human immunodeficiency virus (HIV) induces a persistent and progressive infection, typically leading to the development of acquired immunodeficiency syndrome (AIDS) (Barre-Sinoussi et al., 1983, *Science* 220:868-870; Gallo et al., 1984, *Science* 224:500-503).

Many HIV components have been considered as potential targets for anti-HIV vaccines. For example, the HIV-1 envelope polypeptides (gp160, gp120, gp41) have been shown to be the major antigens for neutralizing anti-HIV antibodies present in AIDS patients (Barin et al., 1985, *Science* 228:1094-1096). Accordingly, these polypeptides are among the most promising antigen candidates. Various portions of gp160, gp120, and/or gp41 have been used as immunogenic targets (see, for example, Ivanoff et al., U.S. Pat. No. 5,141,867; Saith et al., PCT publication WO 92/22654; Shafferman, A., PCT publication WO 91/09872; Formoso et al., PCT publication WO 90/07119). The gp120 envelope glycoprotein has been identified as a particularly attractive target (Emini, E.A. and Putney, S.D., 1992, *Biotechnology* 20:309-326). However, methods and compositions are needed which increase the efficacy of vaccines against HIV.

Genetic vaccination (also referred to as nucleic acid vaccination or DNA vaccination) has been used for inducing immune responses *in vivo*. Injection of cDNA expression cassettes results in *in vivo* expression of the encoded polypeptides (Dubensky et al., 1984, *Proc. Natl. Acad. Sci. USA* 81:7529-7533; Raz et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:4523; Wolff et al., 1990, *Science* 247:1465-1468), with the concomitant development of specific cellular and humoral immune responses directed against the

encoded antigen(s) (Wang et al., 1995, *Hum. Gene Ther.* 6:407-418; Ulmer et al., 1993, *Science* 259:1745-1749; Tang et al., 1992, *Nature* 356:152-154; Michel et al., 1995, *Proc. Natl. Acad. Sci. USA* 92:5307-5311; and Lowrie et al., 1994, *Vaccine* 12:1537-1540). Researchers have employed genetic vaccination to induce humoral and cellular responses to HIV-1 and SIV antigens in macaques and mice (macaques: Wang et al., 1995, *Virology* 221:102-112; Wang et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:4156-4160; and Boyer et al., 1996, *J. Med. Primatol.* 25:242-250; mice: Wang et al., 1995, *Virology* 221:102-112; Lu et al., 1995, *Virology* 209:147-154; Haynes et al., 1994, *AIDS Res. Hum. Retroviruses* 10 (Suppl. 2):S43-S45; Okuda et al., 1995, *AIDS Res. Hum. Retroviruses* 11:933-943).

Recently, Lekutis et al. (1997, *J. Immunol.* 158:4471-4477), assessed the TH cell response elicited by an HIV-1 gp120 nucleic acid vaccine in rhesus monkeys by isolation of gp120-specific, MHC class II-restricted $CD4^+$ T cell lines from the vaccinated animals. The isolated cell lines proliferated in response to APC in the presence of recombinant gp120, as well as to APC expressing HIV-encoded Env polypeptide. The cell lines also responded to Env by secreting IFN-γ and TNF-α without appreciable IL-4 production. These results demonstrate that animals exhibit a cellular immune response to the nucleic acid vaccine.

Similarly, Boyer et al. (1997, *Nature Medicine* 3:625-532) demonstrated that chimpanzees innoculated with an HIV-1 nucleic acid vaccine encoding Env, Rev, and Gag/Pol, developed specific cellular and humoral immune responses to these polypeptides. Further, chimpanzees vaccinated with the nucleic acid vaccine were protected against HIV infection, whereas the control animals were not so protected.

Additionally, Kim et al., (1997 *J. Immunol.* 158:816-826), investigated the role of co-delivery of genes for (1) IL-12 and GM-CSF, and (2) HIV-1 Env and Gag/Pol antigens. Administration of the HIV-1/IL-12 vaccine resulted in a dramatic increase in specific CTL response and in the reduction of specific antibody response. In contrast, administration of the GM-CSF/HIV-1 vaccine resulted in the enhancement of specific antibody response.

Notwithstanding the recent advances in HIV nucleic acid vaccines, there remains a need for compositions and method which enhance the efficacy of a such vaccines.

2.2 Generation of an Immune Response

The introduction of a foreign antigen into an individual elicits an immune response consisting of two major components: (1) a cellular immune response, and (2) a humoral immune response. These responses are mediated by functionally distinct populations of lymphocytes known as T and B cells, respectively (*see generally* Coutinho, 1991, Immune System, *Encyclopedia of Human Biology*, Vol. 4, Ed. Dulbecco, Academic Press, Inc.). T and B cells effect distinct immune responses. One subset of T cells responds to antigen stimulation by producing lymphokines. These lymphokines activate various other immunological cell types. Another T cell subset develops into antigen-specific cytotoxic effector cells, which directly kill antigen-positive target cells. In contrast, B cells attack antigens by producing antibodies which directly bind to and neutralize the antigens.

Cell surface expression of the glycopolypeptide marker CD4 distinguishes helper T cells (TH) from classical cytotoxic T lymphocytes (CTL) and B cells. CD4 T cells are further grouped into functionally distinct subsets based on distinct patterns of lymphokine production (Mosmann and Coffman, 1989, *Ann. Rev. Immunol.* 7:145-173). Type 1 helper T cells (TH1) produce interleukin-2 (IL-2) and γ-interferon (γ-IFN) upon activation. Generally speaking, the lymphokines produced by TH1 cells promote cell-mediated immune responses, augment IgM and IgG2 synthesis by B cells, and activate macrophages (see Stites, D.B., et al., 1994, *Basic and Clinical Immunology*, Appleton & Lange). Type 2 helper T cells (TH2) produce IL-4 and IL-5. The lymphokines produced by TH2 cells generally regulate B cell proliferation and differentiation, synthesis of IgG1 and IgE, and antibody class switching.

Cytotoxic T lymphocytes (CTL) express the CD8 surface marker. Unlike most TH cells, CTLs respond to antigen recognition by directly lysing target cells. CTLs play a prominent role in responding to viral infections and cancer where an antibody response alone is typically inadequate.

Both T and B cell responses exhibit exquisite specificity for the immunizing antigen; however, the mechanisms for antigen recognition differ between these two cell types. B cells recognize antigens by antibodies, either acting as cell surface receptors or as secreted polypeptides, which bind directly to antigens on a solid surface or in solution. In

contrast, T cells only recognize antigens that have been processed or degraded into small fragments and presented on a solid phase such as the surface of antigen-presenting cells (APC). Additionally, antigenic fragments must be presented to T cells in association with major histocompatibility complex (MHC)-encoded class I or class II molecules.

The MHC is a cluster of genes that encodes polypeptides with diverse immunological functions. Class I MHC products are present on all somatic cells. Class II gene products are typically expressed on cells of various hematopoietic lineages and are involved in cell-cell interactions in the immune system.

MHC-encoded polypeptides function as receptors for processed antigenic fragments on the surface of APCs (Bjorkman et al., 1987, *Nature* 329:506-512). The interaction between a T cell and an antigenic fragment occurs only if the antigen is presented by self-MHC. This phenomenon is known as self-MHC restriction of T cells.

The specificity of T cell immune responses for antigens is a function of the unique receptors expressed by these cells. The T cell receptor (TCR) is structurally homologous to an antibody—a heterodimer composed of disulfide-linked glycopolypeptides. Four TCR polypeptide chains known as α, β, γ, and δ have been identified, although the vast majority of functional T cells express the $\alpha\beta$ heterodimeric TCR. Transfer of α and β genes alone into recipient cells is both necessary and sufficient to confer antigen specificity and MHC-restriction (Dembic et al., 1986, *Nature* 320:232-238). Thus, the $\alpha\beta$ TCR appears to be responsible for recognizing a combination of antigenic fragment and MHC determinants.

In summary, the generation of an immune response begins with the sensitization of $CD4^+$ and $CD8^+$ T cell subsets through their interaction with APCs that express MHC-class I or class II molecules associated with antigenic fragments. The sensitized or primed $CD4^+$ T cells produce lymphokines that participate in the activation of B cells and various T cell subsets. The sensitized $CD8^+$ T cells increase in numbers in response to lymphokines and are capable of destroying any cells that express the specific antigenic fragments associated with matching MHC-encoded class I molecules. For example, in the course of a viral infection, CTL eradicate virally-infected cells, thereby limiting the progression of virus spread and disease development.

The presentation of antigens to T cells is carried out by specialized cell populations referred to as antigen presenting cells (APC). Typically, APCs include macrophages/monocytes, B cells, and bone marrow derived dendritic cells (DC). APCs internalize exogenous antigens, cleave them into smaller fragments in enzyme-rich vesicles, and couple the fragments to MHC-encoded products for expression on the cell surface (Goldberg and Rock, 1992, *Nature* 357:375-379). Since APCs express both MHC-encoded class I and class II glycopolypeptides, they can present antigenic fragments to both $CD4^+$ and $CD8^+$ T cells for the initiation of an immune response.

APCs present antigens to T cells with antigen-specific receptors and provide the signals necessary for T cell activation. These signals are incompletely defined, but probably involve a variety of cell surface molecules as well as cytokines or growth factors. The factors necessary for the activation of naive or unprimed T cells may be different from those required for the re-activation of previously primed memory T cells. The ability of APCs to present antigens and deliver signals for T cell activation is commonly referred to as an accessory cell function. Although monocytes and B cells have been shown to be competent APCs, their antigen presenting capacities *in vitro* appear to be limited to the re-activation of previously sensitized T cells. Hence, they are not capable of directly activating functionally naive or unprimed T cell populations.

2.3 Chemokines

Chemokines, or chemoattractant cytokines, are a subgroup of immune factors that mediate chemotactic and other pro-inflammatory phenomena (*see*, Schall, 1991, *Cytokine* 3:165-183). Chemokines are small molecules of approximately 70-80 residues in length. Several chemokine families have been identified, including the CXC (α), CC (β), CX_3C (δ) and C (γ) families, distinguished by the spacings of the N-terminal cysteine residues (Mackay, 1997; Wells and Peitsch, 1997). The N-terminal cysteines of α-chemokines are separated by one amino acid residue (C-X-C). In the β chemokine family, the cysteines are adjacent (C-C) (Clark-Lewis et al., 1995). Members of the α family are chemotactic for neutrophils and lymphocytes, whereas the β family chemokines are chemotactic for monocytes, lymphocytes and eosinophils (Teran and Davies, 1996). Eotaxin is an eosinophil-selective β chemokine which is apparently unique among the chemokines in binding specifically to only one receptor, the CCR-3 receptor (Jose et al., 1996). The only

C, or γ, chemokine identified to date is lymphotactin which has only one pair of cysteines (Kelner et al., 1994; Kennedy et al., 1995). The CX_3C or δ chemokines include neurotactin, a type I membrane-anchored protein which is chemotactic for neutrophils and is upregulated in the microglia of mice with experimental autoimmune encephalomyelitis (Pan et al., 1997), and fractalkine, a membrane-bound molecule with a chemokine domain perched on a mucin-like stalk and which engages in juxtacrine signaling (Premack and Schall, 1996).

There remains a need in the art for compositions and methods which enable the use of the natural chemotactic properties of chemokines for enhancing an immune response.

3. SUMMARY OF THE INVENTION

In one aspect, the present invention provides a purified chimeric polypeptide comprising: (a) one or more chemokine polypeptides selected from the group consisting of (i) chemokines and (ii) polypeptides within one or more of the following groups: fragments of chemokines, analogues of chemokines, derivatives of chemokines, and truncation isoforms of chemokines; (b) one or more antigenic polypeptides; and (c) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptide(s) (hereinafter referred to as "chimeric polypeptides").

In a related aspect, the present invention provides a polynucleotide comprising a nucleotide sequence encoding a chimeric polypeptide comprising: (a) one or more chemokine polypeptides selected from the group consisting of (i) chemokines and (ii) polypeptides within one or more of the following groups: fragments of chemokines, analogues of chemokines, derivatives of chemokines, and truncation isoforms of chemokines; (b) one or more antigenic polypeptides; and (c) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptide(s).

In a narrower aspect, the present invention provides a chimeric polypeptide having the formula: C-L-A; wherein: C is a chemokine polypeptide selected from the group consisting of: (1) chemokines; and (2) polypeptides within one or more of the following groups: chemokine fragments, chemokine analogues, chemokine derivatives, and chemokine

truncation isoforms; A is an antigenic polypeptide; L is a polypeptide linker which does not eliminate the biological activity of C or the antigenicity of A; and C, L and A are joined by peptide bonds. The present invention also provides a nucleic acid encoding a chimeric polypeptide having the formula: **C-L-A** as defined above.

Additionally, the present invention provides a method to enhance an immune response, the method comprising the step of administering to a subject, in an amount effective to produce an immune response, a purified chimeric polypeptide comprising: (a) one or more chemokine polypeptides selected from the group consisting of (i) chemokines and (ii) polypeptides within one or more of the following groups: fragments of chemokines, analogues of chemokines, derivatives of chemokines, and truncation isoforms of chemokines; (b) one or more antigenic polypeptides; and (c) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptide(s).

Furthermore, the present invention provides a method for enhancing an immune response, the method comprising the step of administering to a subject, in an amount effective to produce an immune response, a polynucleotide comprising a nucleotide sequence encoding a chimeric polypeptide comprising: (a) one or more chemokine polypeptides selected from the group consisting of (i) chemokines and (ii) polypeptides within one or more of the following groups: fragments of chemokines, analogues of chemokines, derivatives of chemokines, and truncation isoforms of chemokines; (b) one or more antigenic polypeptides; and (c) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptide(s).

The present invention also provides a pharmaceutical composition for enhancing an immune response, the pharmaceutical composition comprising a pharmaceutically acceptable carrier in association with one or more chimeric polypeptides, each comprising: (a) one or more chemokine polypeptides selected from the group consisting of (i) chemokines and (ii) polypeptides within one or more of the following groups: fragments of chemokines, analogues of chemokines, derivatives of chemokines, and truncation isoforms of chemokines; (b) one or more antigenic polypeptides; and (c) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptide(s).

Additionally, the present invention provides a pharmaceutical composition for enhancing an immune response comprising a pharmaceutically acceptable carrier in association with a polynucleotide comprising a nucleotide sequence encoding a chimeric polypeptide comprising: (a) one or more chemokine polypeptides selected from the group consisting of (i) chemokines and (ii) polypeptides within one or more of the following groups: fragments of chemokines, analogues of chemokines, derivatives of chemokines, and truncation isoforms of chemokines; (b) one or more antigenic polypeptides; and (c) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptide(s).

Further, the present invention provides a method of producing one or more chimeric polypeptides comprising (1) preparing an expression vector comprising a nucleotide sequence encoding a chimeric polypeptide comprising (a) one or more chemokine polypeptides selected from the group consisting of (i) chemokines and (ii) polypeptides within one or more of the following groups: fragments of chemokines, analogues of chemokines, derivatives of chemokines, and truncation isoforms of chemokines; (b) one or more antigenic polypeptides; and (c) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptide(s); (2) transforming a host cell with the expression vector of (1); and (3) causing the host cell to express the chimeric polypeptide.

The chimeric polypeptide may also comprise a signal peptide which is cleavable from the fusion polypeptide, e.g., by enzymatic cleavage.

The invention also provides assays, both *in vitro* and *in vivo*, for testing the efficacy of the therapeutics of the invention.

Other objects and further scope of the present invention will become apparent from the detailed description given hereafter. It should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only; various changes and modifications will become apparent to those skilled in the art from this detailed description.

3.1 Definitions

The word "transform" is broadly used herein to refer to introduction of an exogenous polynucleotide sequence into a prokaryotic or eukaryotic cell by any means known in the art (including, for example, direct transmission of a polynucleotide sequence from a cell or virus particle as well as transmission by infective virus particles), resulting in a permanent or temporary alteration of genotype and in an immortal or non-immortal cell line.

The terms "peptide," "polypeptide," and "protein" are used interchangeably herein and are intended to refer to amino acid sequences of any length.

The term "functional equivalent" is used herein to refer to a polypeptide which is an active analogue, derivative, fragment, truncation isoform or the like of a native polypeptide. A polypeptide is active when it retains some or all of the biological activity of the corresponding native polypeptide.

The terms "antigen" and "antigenic" as used herein are meant to describe a substance that induces an immune response when presented to immune cells of an organism. An antigen may comprise a single immunogenic epitope, or a multiplicity of immunogenic epitopes recognized by a B-cell receptor (i.e., antibody on the membrane of the B cell) or a T-cell receptor. Thus, as used herein, these terms refer to any substance capable of eliciting an immune response, e.g., Human Immunodeficiency Virus (HIV) antigens, Hepatitis virus antigens (HCV, HBV, HAV), *Toxoplasmosis gondii*, Cytomegalovirus, *Helicobacter pylori*, Rubella, and the like, as well as and haptens which may be rendered antigenic under suitable conditions known to those of skill in the art.

The term "therapeutically effective amount" as used in the invention is meant to describe that amount of antigen which induces an antigen-specific immune response. Such induction of an immune response may provide a treatment such as, for example, immunoprotection, immunosuppression, modulation of autoimmune disease, potentiation of cancer immunosurveillance, or vaccination against an infectious disease caused by a pathogen.

The term "immunizingly effective" is used herein to refer to an immune response which confers immunological cellular memory upon the subject of such immune response, with

the effect that a subsequent secondary response (to the same or a similar immunogen) is characterized by one or more of the following: shorter lag phase in comparison to the lag phase resulting from a corresponding exposure in the absence of immunization; production of antibody which continues for a longer period than production of antibody for a corresponding exposure in the absence of such immunization; a change in the type and quality of antibody produced in comparison to the type and quality of antibody produced from such an exposure in the absence of immunization; a shift in class response, with IgG antibodies appearing in higher concentrations and with greater persistence than IgM; an increased average affinity (binding constant) of the antibodies for the antigen in comparison with the average affinity of antibodies for the antigen from such an exposure in the absence of immunization; and/or other characteristics known in the art to characterize a secondary immune response.

As used herein, "pharmaceutically acceptable" component (such as a salt, carrier, excipient or diluent) of a formulation according to the present invention is a component which (1) is compatible with the other ingredients of the formulation in that it can be combined with the chimeric polypeptides of the present invention without eliminating the capacity of the chimeric polypeptides to enhance and/or stimulate an immunizingly effective immune response in a subject; and (2) is suitable for use with animals (including humans) without undue adverse side effects (such as toxicity, irritation, and allergic response). Side effects are "undue" when their risk outweighs the benefit provided by the pharmaceutical composition. Examples of pharmaceutically acceptable components include, without limitation, any of the standard pharmaceutical carriers such as phosphate buffered saline solutions, water, emulsions such as oil/water emulsion, microemulsions and various types of wetting agents.

As used herein, the term "native" used in reference to a polypeptide, such as an antigen polypeptide or a chemokine polypeptide, is used to indicate that the polypeptide indicated has the amino acid sequence of the corresponding polypeptide as found in nature.

As used herein, the terms "hinge region" and "Ig hinge region" refer to a polypeptide comprising an amino acid sequence that shares sequence identity, or similarity, with a portion of a naturally occurring Ig hinge region sequence. Sequence similarity of the

hinge region linkers with naturally occurring Ig hinge region amino acid sequences can range from at least 50% to about 75%-80%, and typically greater than about 90%.

4. Brief Description of Nucleotide Sequences

SEQ ID NO: 1 is the DNA sequence encoding the chimeric polypeptide of Example 3 (SEQ ID NO: 2).

SEQ ID NO: 2 is the amino acid sequence of Example 3. Amino acids 1-24 constitute the signal peptide. Amino acids 25-92 constitute murine MDC. Amino acids 93-105 constitute the hinge region of Ig gamma 2a/b as reported for mouse strain C57BL/6. Amino acids 106-477 constitute the p24 core antigen of HIV-1, strain IIIB. The mature peptide includes amino acids 25-473.

SEQ ID NO: 3 is the DNA sequence encoding the chimeric polypeptide of Example 2 (SEQ ID NO: 4).

SEQ ID NO: 4 is the amino acid sequence of Example 2. Amino acids 1-16 constitute the signal peptide. Amino acids 17-107 constitute murine BLC. Amino acids 108-120 constitute the hinge region of Ig gamma 2a/b as reported for mouse strain C57BL/6. Amino acids 121-490 constitute the p24 core antigen of HIV-1, strain 111B. The mature peptide includes amino acids 17-490.

SEQ ID NO: 5 is the DNA sequence encoding the chimeric polypeptide of Example 1 (SEQ ID NO: 6).

SEQ ID NO: 6 is the amino acid sequence of Example 1. Amino acids 1-23 constitute the signal peptide. Amino acids 24-91 constitute murine RANTES. Amino acids 92-104 constitute the hinge region of Ig gamma 2a/b as reported for mouse strain C57BL/6. Amino acids 105-472 constitute the p24 core antigen of HIV-1, strain IIIB. The mature peptide includes amino acids 24-472.

SEQ ID NO: 7 is the DNA sequence encoding the chimeric polypeptide of Example 4 (SEQ ID NO: 8).

SEQ ID NO: 8 is the amino acid sequence of Example 4. Amino acids 1-23 constitute the signal peptide. Amino acids 24-148 constitute murine MCP-1/JE. Amino acids 149-161 constitute the hinge region of Ig gamma 2a/b as reported for mouse strain C57BL/6. Amino acids 162-528 constitute the p24 core antigen of HIV-1, strain IIIB. The mature peptide includes amino acids 24-528.

5. Detailed Description of the Invention

The present invention relates generally to chimeric polypeptides for use in inducing an immune response in humans or other animals. The immune response is preferably immunizingly effective, as defined herein.

In one aspect, the chimeric polypeptides of the present invention generally comprise: (a) one or more chemokine polypeptides selected from the group consisting of (i) chemokines and (ii) polypeptides within one or more of the following groups: fragments of chemokines, analogues of chemokines, derivatives of chemokines, and truncation isoforms of chemokines; (b) one or more antigenic polypeptides; and (c) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptide(s). In a preferred mode, the chimeric polypeptides of the present invention consist of or consist essentially of: (a) one or more chemokine polypeptides selected from the group consisting of (i) chemokines and (ii) polypeptides within one or more of the following groups: fragments of chemokines, analogues of chemokines, derivatives of chemokines, and truncation isoforms of chemokines; (b) one or more antigenic polypeptides; and (c) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptide(s).

The present invention also relates to a method for vaccinating a subject and/or enhancing the efficacy of a vaccine, in a subject, said method comprising administering to the subject a chimeric polypeptide of the present invention.

The present invention provides for therapeutic compositions comprising (1) chimeric polypeptides and/or nucleic acids encoding such chimeric polypeptides; and (2) a pharmaceutically acceptable carrier.

In another preferred embodiment of the invention, nucleic acid sequences that encode chimeric polypeptides of the present invention are provided. In a preferred mode, the nucleic acid sequences are operatively linked to gene regulatory sequences capable of directing the expression of the chimeric polypeptides upon introduction into a host (as in DNA or RNA vaccination) or upon introduction to a suitable cell (e.g., the cell of a subject). In another aspect, the present invention comprises methods for vaccinating a subject and/or vaccinating, and/or enhancing the efficacy of a vaccine, in a subject, comprising the step of administering such nucleic acid sequences and/or such cells to a subject.

The present invention also provides methods for making the chimeric polypeptide of the invention. For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

5.1 Chimeric Polypeptides and Methods for Administering Same

The chimeric polypeptides of the present invention comprise (a) one or more chemokine polypeptides selected from the group consisting of (i) chemokines and (ii) polypeptides within one or more of the following groups: fragments of chemokines, analogues of chemokines, derivatives of chemokines, and truncation isoforms of chemokines; (b) one or more antigenic polypeptides; and (c) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptide(s).

In one aspect, the methods of the present invention comprise the step of administering to a subject an immunizingly effective amount of one or more of the chimeric polypeptides of the present invention. Such methods confer immunization to one or more antigens of the chimeric polypeptides against which an immune response is desired. Preferably the antigen(s) are associated with a disease or condition to which the subject is susceptible; however, such susceptibility is not strictly necessary, as the chimeric polypeptides of the present invention also have utility in the production of antibodies for commercial use and/or for further study.

The compositions of the present invention preferably comprise an immunizingly effective

amount of one or more chimeric polypeptides of the present invention along with a pharmaceutically acceptable carrier.

The methods and compositions of the present invention may be used to elicit a humoral and/or a cell-mediated response against the antigen(s) of the vaccine in a subject. In one specific embodiment, the methods and compositions elicit a humoral response against the administered antigen(s) in a subject. In another specific embodiment, the methods and compositions elicit a cell-mediated response against the administered antigen(s) in a subject. In a preferred embodiment, the methods and compositions elicit both a humoral and a cell-mediated response.

The subjects to which the present invention is applicable are preferably mammalian or vertebrate species, e.g., cows, horses, sheep, pigs, fowl (*e.g.*, chickens), goats, cats, dogs, hamsters, mice and rats, monkeys, rabbits, chimpanzees, and humans. In a preferred embodiment, the subject is a human. The compositions and methods of the invention can be used to either prevent a disease or disorder, or to treat a particular disease or disorder, where an immune response against a particular antigen or antigens is effective to treat or prevent the disease or disorder. Such diseases and disorders include, but are not limited to, viral infections, such as HIV, CMV, hepatitis, herpes virus, measles, etc, bacterial infections, fungal and parasitic infections, cancers, and any other disease or disorder amenable to treatment or prevention by eliciting an immune response against a particular antigen or antigens.

In a preferred embodiment, the subject is infected or at risk of being infected with HIV virus. Thus, the invention provides methods and compositions to enhance the efficacy of an HIV vaccine. Such a vaccine can be administered to prevent, or treat HIV, and/or reduce the rate of onset or progression of HIV.

In a specific embodiment, a chimeric polypeptide containing a chemokine and/or functional equivalent of a chemokine is joined via a peptide bond to an antigen against which immunity or elicitation of an immune response is desired. In a preferred embodiment, the chimeric polypeptide containing the chemokine and/or its functional equivalent is joined via a flexible polypeptide linker to all or a portion of an antigen.

The various components of the chimeric polypeptides (chemokine polypeptides, polypeptide linkers, and antigen polypeptides) are described in the ensuing sections.

5.1.1 Chemokines Polypeptides

The chimeric polypeptides of the present invention comprise one or more chemokine polypeptides. These chemokine polypeptides comprise one or more chemokines and/or one or more functional equivalents of chemokines.

In a preferred aspect, the chemokine polypeptide is selected from the chemokines of Table 1.

Table 1

Chemokine Class	Chemokines	Abbreviations	Accession Number
CC chemokines (continued)	Lymphocyte and Monocyte chemoattractant; Monotactin	LMC	AF055467
	Activation-induced, chemokine-related molecule	ATAC	x86474
	Myeloid progenitor inhibitory factor-1	MPIF-1; MIP-3 or ckbeta8	u85767
	Myeloid progenitor inhibitory factor-2	MPIF-2	u85768
	Stromal cell-derived factor 1 alpha	SDF-1α; PBSF	L36034
CXC chemokines	Stromal cell-derived factor 1 beta	SDF-1β; PBSF	L36033
	B-cell-attracting chemokine 1	BLC	AJ002211
	HuMIG		x72755 s60728
	H174		AF002985
	Interferon-stimulated T-cell alpha chemoattractant	I-TAC	AF030514
	Interleukin-8	IL-8	m17017; y00787
	IP-10		X02530
	platelet factor 4	PF4	M20901
	growth-regulated gene-alpha	GRO-α	J03561
	growth-regulated gene-beta	GRO-β	M36820
	growth-regulated gene-gamma	GRO-γ	M36821
	Neutrophil-activating protein 2	NAP-2; CTAP-3	M54995; M38441
	ENA-78		L37036
	Granulocyte chemotactic protein 2	GCP-2	Y08770
C-chemokines	lymphotactin (-ATAL)	SCM-1	D63789; D63790; x86474
CX_3C-chemokines	Fractalkine/neurotactin		U91835 U84487

The chemokine polypeptides of the present invention may also be selected from the group consisting of: Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, Activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2

alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EBI1-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, Liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, Thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, Platelet factor 4, Growth-regulated gene-alpha, Growth-regulated gene-beta, Growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, granulocyte chemotactic protein 2, lymphotactin, and Fractalkine/neurotactin, and viral chemokines.

Viral chemokines are an integral part of the genome of many viruses. Examples of viral chemokines having utility in the chimeric polypeptides of the present invention are: from HHV-5 or CMV, US22 (Acc. # 1139602); from HHV6, 2 unnamed chemokines (genome Acc. # x83413); from HHV-8, vMIP-1 and vMIP-II (genome Acc. # KSU75698); from HHV-7, unnamed chemokine homologous to US22 of CMV; from human poxvirus, molluscum contagiosum virus, type 1, (Acc. # u86945) and type 2 (Acc # u95749), both of which remain unnamed.

Chemokines useful in the practice of the present invention include, but are not limited to, chemokines from eukaryotes, prokaryotes, and viruses; preferred species include mice, hamsters, dogs, cats, monkeys, rabbits, chimpanzees and humans. In one preferred embodiment, the chemokine(s) are of human origin.

Any chemokine or functional equivalent thereof that, as a component of a chimeric polypeptide of the present invention, is capable upon administration of enhancing an immune response, as compared to the immune response generated in the absence of administration of such chimeric polypeptide, can be used in the methods and compositions of the present invention.

In one specific embodiment, the chemokine polypeptide comprises a purified full-length chemokine. Alternatively, the chemokine polypeptide may comprise a functional equivalent of a chemokine. Further, the chimeric polypeptides of the invention may comprise multiple chemokines and/or functional equivalents of chemokines.

In another embodiment, the chemokine polypeptide(s) of the chimeric polypeptides of the present invention are purified derivatives of the native chemokines. Such derivatives typically have one or more insertions of, or substitutions with, one or more non-classical amino acids relative to a native chemokine. These derivatives will, when administered as a component of a chimeric polypeptide of the present invention, elicit an enhanced immune response, enhance an immune response and/or cause an immunizingly effective response. In a preferred aspect, the purified derivative has only one or more conservative substitutions in sequence relative to a native chemokine and retains the capacity to enhance the elicitation of an immune response, elicit an enhanced immune response, enhance an immune response and/or cause an immunizingly effective response. Chemokines and functional equivalents thereof useful in the chimeric polypeptides of the present invention may be derived from any suitable source and obtained by any method known in the art.

Preferably, the chemokine is of the same species as the subject to which the vaccine is administered. In a preferred embodiment, a human chemokine is administered to a human subject.

In another embodiment, chemokine polypeptide(s) of the chimeric polypeptides of the present invention comprise a chemokine or functional equivalent thereof having deletional, insertional and/or substitutional mutations. Such chimeric polypeptides retain some or all of the activity of the corresponding native chemokine to elicit an enhanced immune response, enhance an immune response and/or cause an immunizingly effective response.

5.1.2 Antigens

The chimeric polypeptides of the present invention comprise one or more antigen polypeptides. In one aspect, the chimeric polypeptides comprise one or more of the same

antigen species. In another aspect, the chimeric polypeptides comprise two or more different antigen species.

The antigen polypeptide(s) may comprise any antigen(s), including fragments, analogues, derivatives and other functional equivalents of antigens. For example, antigens useful in the practice of the present invention include polypeptides which are antigenic in mice, hamsters, dogs, cats, monkeys, rabbits, chimpanzees and humans. In one preferred embodiment, the antigen polypeptide(s) are antigenic to humans. Suitable antigen polypeptides may, for example, be selected from the group consisting of plants, fungi, protozoa, bacteria, and viruses. Suitable antigen polypeptides also include self-antigens, allergens and tumor-associated antigens. In a preferred aspect, the antigen polypeptides comprise antigens and/or epitopes from a bacterium or virus pathogenic to humans. Further, nonlimiting examples of antigens suitable for use in the chimeric polypeptides of the present invention are antigens which can elicit an enhanced immune response, enhance an immune response and/or cause an immunizingly effective response to the following diseases and disease-causing agents: adenoviruses; anthrax; Bordetella pertussus; Botulism; bovine rhinotracheitis; Branhamella catarrhalis; canine hepatitis; canine distemper; Chlamydiae; Cholera; coccidiomycosis; cowpox; cytomegalovirus; Dengue fever; dengue toxoplasmosis; Diphtheria; encephalitis; Enterotoxigenic E. coli; Epstein Barr virus; equine encephalitis; equine infectious anemia; equine influenza; equine pneumonia; equine rhinovirus; Escherichia coli; feline leukemia; flavivirus; Globulin; haemophilus influenza type b; Haemophilus influenzae; Haemophilus pertussis; Helicobacter pylori; Hemophilus; hepatitis; hepatitis A; hepatitis B; Hepatitis C; herpes viruses; HIV; HIV-1 viruses; HIV-2 viruses; HTLV; Influenza; Japanese encephalitis; Klebsiellae species; Legionella pneumophila; leishmania; leprosy; lyme disease; malaria immunogen; measles; meningitis; meningococcal; Meningococcal Polysaccharide Group A; Meningococcal Polysaccharide Group C; mumps; Mumps Virus; mycobacteria; Mycobacterium tuberculosis; Neisseria; Neisseria gonorrhoeae; Neisseria meningitidis; ovine blue tongue; ovine encephalitis; papilloma; parainfluenza; paramyxovirus; paramyxoviruses; Pertussis; Plague; Pneumococcus; Pneumocystis carinii; Pneumonia; Poliovirus; Proteus species; Pseudomonas aeruginosa; rabies; respiratory syncytial virus; rotavirus; Rubella; Salmonellae; schistosomiasis; Shigellae; simian immunodeficiency virus; Smallpox; Staphylococcus aureus; Staphylococcus species; Streptococcus pneumoniae; Streptococcus pyogenes; Streptococcus species;

swine influenza; tetanus; Treponema pallidum; Typhoid; Vaccinia; varicella-zoster virus; and Vibrio cholerae.

Preferred antigens are those which are known in the art to be useful in as components of vaccines. The antigens may, for example, include various toxoids, viral antigens and/or bacterial antigens. For example, the antigens may include antigens commonly employed in the following vaccines: chickenpox vaccine; diphtheria, tetanus, and pertussis vaccines; haemophilus influenzae type b vaccine (Hib); hepatitis A vaccine; hepatitis B vaccine; influenza vaccine; measles, mumps, and rubella vaccines (MMR); pneumococcal vaccine; polio vaccines; rotavirus vaccine; anthrax vaccines; and tetanus and diphtheria vaccine (Td).

The chimeric polypeptides and compositions of the present invention may be used to enhance an immune response to infectious agents (e.g., bacteria, parasites, fungi and viruses) and/or their toxoids, as well as diseased or abnormal cells (e.g., tumors, cancers and other neoplasms). The chimeric polypeptides and compositions of the invention may be used to treat or prevent a disease or disorder amenable to treatment or prevention by generating an immune response to the antigen polypeptides of the chimeric polypeptides and compositions. In one preferred embodiment, the antigen polypeptides are polypeptides encoded by any genes of the HIV genome including the *env, gag, pol, nef, vif, rev,* and *tat* genes, or functional equivalents of such polypeptides. In a more preferred embodiment, the antigen is an HIV-associated gp120 polypeptide or p24 polypeptide.

Nucleic acids of the present invention can also encode chimeric polypeptides comprising an antigen having deletional, insertional or substitutional mutations or combinations thereof, which derivative retains the capacity to elicit an enhanced immune response, enhance an immune response and/or cause an immunizingly effective response.

The antigen polypeptide may also be a hapten, *i.e.*, a molecule that is antigenic in that it can react selectively with cognate antibodies, but is also non-immunogenic in that it cannot elicit an immune response. In such circumstances, the hapten can be rendered immunogenic by its inclusion in the chimeric polypeptide of the present invention. Alternatively, the chimeric polypeptide may be attached to a large polypeptide, such as serum albumin, to confer immunogenicity to a hapten. The chimeric polypeptide

comprising a hapten attached to a large polypeptide may be formulated for use as a vaccine.

5.1.3 Polypeptide Linkers

In preferred aspects of the present invention, a polypeptide linker sequence is incorporated into the chimeric protein construct by well-known standard molecular biology techniques (e.g., PCR). The linker sequence is used to separate the chemokine and the antigen domains by a distance sufficient to ensure that each domain assumes its biologically active conformation. Suitable polypeptide linker sequences (1) will adopt a flexible extended conformation, (2) will not exhibit a propensity for developing a secondary structure that could interact with the functional chemokine and antigen domains, and (3) will have minimal hydrophobic or charged character that could promote interaction with the functional protein domains.

Preferred linker sequences are non-immunogenic, and for this reason are preferably selected from sequences found in proteins from the species to which the chimeric polypeprtide is to be administered, i.e. human protein sequences for chimeric constructs intended for delivery to a human host, or from non-immunogenic synthetic sequences.

The length of the polypeptide linker sequence may vary without significantly affecting the biological activity of the chimeric protein. The polypeptide linker preferably ranges from 2 to 30 amino acid residues in length, suitably, from 3 to 28 amino acid residues in length, and more suitably from 5 to 28 amino acids in length.

The appropriate linker may readily be selected by the practitioner from the many available in the art based on the above-mentioned three criteria. An additional consideration for the selection of an appropriate linker is the particular properties of the chemokine polypeptide and antigen polypeptide being joined. Polypeptide linkers that have been described in the published literature and which may have utility in the construction of the chimeric proteins of the invention are described below.

Typical surface amino acids in flexible protein regions include Gly, Asn and Ser. Virtually any permutation of amino acid sequences containing Gly, Asn and Ser would be expected

to satisfy the above criteria for a polypeptide linker sequence. Other near neutral amino acids, such as Thr and Ala, also may be used in the linker sequence. Thus, amino acid sequences useful as linkers of chemokine and antigen include the $Gly_4SerGly_5Ser$ linker (GGGGSGGGGGS, SEQ ID NO: 19) used in U.S. Pat. No. 5,108,910 or a series of four (AlaGlySer) residues (AGSAGSAGSAGS, SEQ ID NO: 20). Still other amino acid sequences that may be used as linkers are disclosed in Maratea et al., Gene 40: 39-46 (1985); Murphy et al., Proc. Nat'l. Acad. Sci. USA 83: 8258-62 (1986); U.S. Pat. No. 4,935,233; and U.S. Pat. No. 4,751,180. Such polypeptide linkers are reported to be useful in the construction of fusion proteins comprising granulocyte macrophage colony-stimulating-factor (GM-CSF) and antigens (U.S. Pat. No. 5,616,477).

Such "Gly-Ser" linkers are advantageous in that they may be constructed such that the polypeptide linker itself is non-immunogenic. For example, in U.S. Pat. Nos. 5,723,125 and 5,908,626, hybrid proteins comprising interferon linked to a human immunoglobulin Fc fragment are described, wherein the linker is a polypeptide of sequence GGSGGSGGGGSGGGGS (SEQ ID NO: 21).

There has long been an interest in constructing single chain hybrid polypeptides made up of the variable binding regions from the light and heavy chains of an immunoglobulin for use as therapeutics and research tools. The types of polypeptide linkers employed were reviewed in U.S. Pat. No. 5,856,456, as summarized below. Such linkers are flexible and of an appropriate length and hence have utility in the practice of the present invention.

The general considerations behind the design and construction of polypeptide linkers for use in the construction of such single chain hybrid polypeptides have been described in U.S. Pat. No. 4,946,778 (Ladner et al.). Computer design of linkers has also been described in U.S. Pat. Nos. 4,704,692, 4,853,871, 4,908,773 and 4,936,666.

Four linkers are described in the '778 disclosure: TRY40, TRY59, TRY61, and TRY104b. TRY40 is a double linker with 3- and 7-amino acid sequences comprising the linkers. The sequences are PGS (SEQ ID NO: 22) and IAKAFKN (SEQ ID NO: 23). TRY59 is an 18-residue single linker having the sequence KESGSVSSEQLAQFRSLD (SEQ ID No. 24). TRY61 is a 14-residue single linker having the sequence VRGSPAINVAVHVF (SEQ ID NO: 25). TRY104b is a 22-residue single linker constructed primarily of a helical segment

from human hemoglobin. The sequence is AQGTLSPADKTNV KAAWGKVMT (SEQ ID NO: 26).

Traunecker et al., EMBO J. 10(12):3655-3659 (1991) disclosed an 18-amino acid linker for joining the first two N-terminal CD4 domains and the combining site of the human CD3 complex. Its sequence is VEGGSGGSGGSGGSGGVD (SEQ ID NO: 27). The final bispecific single-chain polypeptide is called Janusin, and targets cytotoxic lymphocytes on HIV-infected cells.

Fuchs et al., Bio/Technology 9:1369-1372 (1991), used an 18-residue linker to join the heavy- and light-chain variable domains of a humanized antibody against chick lysozyme. The 18-residue linker was partially derived from alpha-tubulin and contains a MAb epitope specific to alpha-tubulin. The full sequence is GSASAPKLEEGEFSEARE (SEQ ID NO: 28).

As summarized in U.S. Pat. No. 5,856,456, many single chain hybrid polypeptides made up of the variable binding regions from the light and heavy chains of an antibody have been disclosed in the literature: Huston, J. S. et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); Huston, J. S. et al., SIM News 38(4) (Suppl.):11 (1988); McCartney, J. et al., ICSU Short Reports 10:114 (1990); Nedelman, M. A. et al., J. Nuclear Med. 32 (Suppl.):1005 (1991); Huston, J. S. et al., In: Molecular Design and Modeling: Concepts and Applications, Part B, edited by J. J. Langone, Methods in Enzymology 203:46-88 (1991); Huston, J. S. et al., In: Advances in the Applications of Monoclonal Antibodies in Clinical Oncology, Epenetos, A. A. (Ed.), London, Chapman & Hall (1993); Bird, R. E. et al., Science 242:423-426 (1988); Bedzyk, W. D. et al., J. Biol. Chem. 265:18615-18620 (1990); Colcher, D. et al., J. Nat. Cancer Inst. 82:1191-1197 (1990); Gibbs, R. A. et al., Proc. Natl. Acad. Sci. USA 88:4001-4004 (1991); Milenic, D. E. et al., Cancer Research 51:6363-6371 (1991); Pantoliano, M. W. et al., Biochemistry 30:10117-10125 (1991); Chaudhary, V. K. et al., Nature 339:394-397 (1989); Chaudhary, V. K. et al., Proc. Natl. Acad. Sci. USA 87:1066-1070 (1990); Batra, J. K. et al., Biochem. Biophys. Res. Comm. 171:1-6 (1990); Batra, J. K. et al., J. Biol. Chem. 265:15198-15202 (1990); Chaudhary, V. K. et al., Proc. Natl. Acad. Sci. USA 87:9491-9494 (1990); Batra, J. K. et al., Mol. Cell. Biol. 11:2200-2205 (1991); Brinkmann, U. et al., Proc. Natl. Acad. Sci. USA 88:8616-8620 (1991); Seetharam, S. et al., J. Biol. Chem. 266:17376-17381 (1991); Brinkmann, U. et

al., Proc. Natl. Acad. Sci. USA 89:3075-3079 (1992); Glockshuber, R. et al., Biochemistry 29:1362-1367 (1990); Skerra, A. et al., Bio/Technol. 9:273-278 (1991); Pack, P. et al., Biochem. 31:1579-1534 (1992); Clackson, T. et al., Nature 352:624-628 (1991); Clackson, T. et al., Nature 352:624-628 (1991); Marks, J. D. et al., J. Mol. Biol. 222:581-597 (1991); Iverson, B. L. et al., Science 249:659-662 (1990); Roberts, V. A. et al., Proc. Natl. Acad. Sci. USA 87:6654-6658 (1990); Condra, J. H. et al., J. Biol. Chem. 265:2292-2295 (1990); Laroche, Y. et al., J. Biol. Chem. 266:16343-16349 (1991); Holvoet, P. et al., J. Biol. Chem. 266:19717-19724 (1991); Anand, N. N. et al., J. Biol. Chem. 266:21874-21879(1991); Fuchs, P. et al., Bio/Technol. 9:1369-1372 (1991); Breitling, F. et al., Gene 104:104-153 (1991); Seehaus, T. et al., Gene 114: in press (1992); Takkinen, K. et al., Prot. Eng. 4:837-841 (1991); Dreher, M. L. et al., J. Immunol. Methods 139:197-205 (1991); Mottez, E. et al., Eur. J. Imunol. 21:467-471 (1991); Traunecker, A. et al., Proc. Natl. Acad. Sci. USA 88:8646-8650 (1991); Traunecker, A. et al., EMBO J. 10:3655-3659 (1991); Hoo, W. F. S. et al., Proc. Natl. Acad. Sci. USA 89:4759-4763 (1993). Linker lengths used in those hybrid polypeptides vary from 10 to 28 residues.

Polypeptide linkers that are resistant to aggregation and proteolysis are desirable. In U.S. Pat. No. 5,856,456, polypeptide linkers are described that incorporate within their sequences a proline residue adjacent to a charged amino acid, preferably lysine or arginine. Proline, lacking amide hydrogens, renders the polypeptide chain resistant to lysis by proteases, e.g., subtilisin. The disclosed polypeptide linkers comprise sequences of amino acids numbering from about 2 to about 50 having a first end connected to a first protein domain, and having a second end connected to a second protein domain, wherein the polypeptide comprises at least one proline residue within the sequence, the proline being positioned next to a charged amino acid, and the charged amino acid-proline pair is positioned within the polypeptide linker to inhibit proteolysis of said polypeptide.

The polypeptide linkers described in U.S. Pat. No. 5,856,456 and also having utility in the practice of the present invention comprise the amino acid sequence:

$$U_mXPZ_n$$

wherein the numbering order from left to right (amino to carboxyl) is up to 50 residues, U and Z can be single natural amino acids, homopolymers of natural amino acids, or

heteropolymers of natural amino acids, such that n and m are any integers from 0 to 48 and n+m is not greater than 48, and X is a charged amino acid. In a preferred embodiment, however, X is lysine or arginine and at least one of the U_m and Z_n sequences comprises at least one alternating glycine-serine sequence. The more preferable polypeptide linker comprises the amino acid sequence:

GSTSGSGXPGSGEGSTKG (SEQ ID NO: 29),

wherein the numbering order from left to right (amino to carboxyl) is 1 to 18, and X is a charged amino acid. In a preferred embodiment, X is lysine or arginine.

The chimeric proteins of the invention can advantageously be formed by joining the chemokine to the antigen via a polypeptide linker which comprises the hinge region of an immunoglobulin. There are five classes of immunoglobulins (IgA, IgD, IgE, IgG, and IgM) in higher vertebrates, all of which contain a hinge region connecting the variable, antigen-binding regions. In addition, some of the classes have subclasses (e.g., IgG_1, IgG_2, IgG_3, IgG_4). The hinge region sequences are highly conserved within species and Ig lineages. The hinge regions function to allow the variable regions to flex and move about to bind with high affinity. The hinge region is often divided into three regions: the upper, middle, and lower hinge. The upper hinge is defined as the number of amino acids between the end of the first domain of the heavy chain and the first cysteine forming an inter-heavy-chain disulfide bond. The middle hinge is high in proline and contains the inter-heavy-chain cysteine disulfide bonds. The lower hinge connects the middle hinge to the C_H2 domain.

Hinge regions useful in the practice of the present invention may comprise the entire hinge region or any combination of the upper, middle or lower hinge regions. Fragments of hinge regions used as the polypeptide linker in the chimeric polypeptides of the present invention, must only be long enough to allow the attached chemokine and antigen to assume and/or retain their respective biologically active conformations.

International Publication No. WO 99/02711 disclosed formation of fusion proteins using immunoglobulin hinge regions as polypeptide linkers. In particular, this publication reported the construction of a homo-fusion protein incorporating two erythropoietin

molecules and having increased biological activity for treatment of anemia. The Ig hinge region sequences or combinations thereof summarized in this publication, are set forth in Table 2:

Table 2

Source	Upper Hinge	Middle Hinge	Lower Hinge
Human IgG_1	EPKSCDKTHT (SEQ ID NO: 30)	CPPCP (SEQ ID NO: 31)	APELLGGP (SEQ ID NO: 32)
Human IgG_2	ERK (SEQ ID NO: 33)	CCVECPPCP (SEQ ID NO: 34)	APPVAGP (SEQ ID NO: 35)
Human IgG_3	ELKTPLGDTTHT (SEQ ID NO: 36)	CPRCP (SEQ ID NO: 37) EPKSCDTPPPCPRCP (SEQ ID NO: 38)	APELLGGP (SEQ ID NO: 39)
Human IgG_3M15	EPKS (SEQ ID NO: 40)	CDTPPPCPRCP (SEQ ID NO: 41)	APELLGGP (SEQ ID NO: 42)
Human IgG_4	ESKYGPP (SEQ ID NO: 43)	CPSCP (SEQ ID NO: 44)	APEFLGGP (SEQ ID NO: 45)
Mouse IgG_1	VPRDCG (SEQ ID NO: 46)	CKPCICT (SEQ ID NO: 47)	VPSEVS (SEQ ID NO: 48)
Mouse IgG_{2A}	EPRGPTIKP (SEQ ID NO: 49)	CPPCKCP (SEQ ID NO: 50)	APNLLGGP (SEQ ID NO: 51)

In another example, the hinge region from human IgG_3 was used to join nerve growth factor (NGF) and transferrin to yield a chimeric protein that retained NGF activity and was actively tranported across the blood-brain barrier (Park et al., J. Drug Target 1998, 6(1):53-64). Xiang et al. (J. Biotechnol. 1997, 53(1):3-12) described the construction of a bifunctional antibody/cytokine fusion protein using the hinge region of the antibody as the connection point for the cytokine.

In a preferred example of the present invention, the hinge region of murine IgG 2a/b (mouse strain C57BL/6) was utilized as the polypeptide linker sequence:

EPRVPITQNPCPP (SEQ ID NO: 52).

Each protein may be attached to the polypeptide linker region via either its amino- or carboxyl-terminus.

The invention also relates to genetic sequences encoding linked fusion polypeptides containing the novel polypeptide linker herein described, methods of making such linked fusion polypeptides, and methods of producing such linked fusion polypeptides, for example, via recombinant DNA technology.

5.2 Nucleic Acids Encoding Chimeric Polypeptides

The present invention also provides a method to enhance the efficacy of a vaccine in a subject, which method comprises administering to a subject a nucleic acid encoding a chimeric polypeptide of the present invention. The nucleic acid may be administered alone (e.g., as a nucleic acid vaccine) or as a component of a cell which has been transformed to express one or more chimeric polypeptides of the present invention.

Expression of the encoded chimeric polypeptide is generally under control of one or more appropriate gene regulatory elements. These regulatory elements can be any regulatory element known in the art which enables expression of the chimeric polypeptide(s) upon introduction of the nucleic acid into a suitable cell (e.g., a cell of the subject). The chimeric polypeptide is preferably expressed in an immunizingly effective amount. It will be appreciated by those of skill in the art that the immunizingly effective dosage will be related both to the rate of expression of the chimeric polypeptide and to the amount of chimeric polypeptide-expressing nucleic acid administered to the subject.

The present invention also provides compositions comprising a purified nucleic acid encoding one or more chimeric polypeptides of the present invention. Upon administeration, the chimeric polypeptide component(s) of the compositions elicit an enhanced immune response, enhance an immune response and/or cause an immunizingly effective response.

Similarly, the present invention also provides compositions comprising a cell transformed to express one or more chimeric polypeptides of the present invention. The nucleic acid used to transform such cell comprises a nucleotide sequence encoding the chimeric

polypeptide(s). This chimeric polypeptide-encoding nuceic acid sequence is operably linked to one or more gene regulatory elements known in the art, thereby enabling expression of the chimeric polypeptides upon introduction of the nucleic acid into the cell.

The methods and compositions of the present invention include any nucleic acid which comprises a nucleotide sequence encoding one or more chimeric polypeptides capable of eliciting an enhanced immune response, enhancing an immune response against an antigen and/or inducing an immunizingly effective immune response.

In a specific embodiment, the nucleotide sequence encoding the chimeric polypeptide(s) comprises a nucleotide sequence selected from the group consisting of the nucleic acid sequences of SEQ ID NOS: 1, 3, 5 and 7. In another embodiment, the nucleotide sequence encodes a polypeptide selected from the group consisting of SEQ ID NOS: 2, 4, 6 and 8. In still another embodiment, the nucleotide sequence encodes a polypeptide selected from the group consisting of: amino acids 25-477 of SEQ ID NO: 2; amino acids 17-493 of SEQ ID NO: 4; amino acids 24-477 of SEQ ID NO: 6; and amino acids 24-528 of SEQ ID NO: 8.

The present invention also provides nucleic acid vaccines and nucleic acid vaccine formulations. These nucleic acid vaccines comprise a nucleotide sequence which expresses a chimeric polypeptide comprising (a) one or more chemokine polypeptides selected from the group consisting of (i) chemokines and (ii) polypeptides within one or more of the following groups: fragments of chemokines, analogues of chemokines, derivatives of chemokines, and truncation isoforms of chemokines; (b) one or more antigenic polypeptides; and (c) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptide(s).

The nucleic acid vaccines are produced by any method known in the art for constructing an expression vector comprising a nucleotide sequence encoding one or more of the chimeric polypeptides of the present invention. Suitable expression vectors comprise promoters, terminators and polyadenylation coding regions to control the expression of the encoded polypeptide and permit expression of the encoded chimeric polypeptides in the subject.

The present invention also provides cellular vector vaccines. These vaccines comprise recombinant cells which have been transformed to express the chimeric polypeptides. Preferred vaccines use eukaryotic expression vectors to produce immunizing polypeptides in the vaccinated host. Cells may be provided to the subject in a living or non-living state as components of a vaccine composition. Suitable cells include any of a variety of eukaryotic and prokaryotic cells. A preferred cell is attenuated *Salmonella typhi.*

The nucleic acid vaccines and cellular vector vaccines of the present invention can be administered by any method known in the art for administration of DNA. Common methods of delivery are intramuscular and intradermal saline injections and gene gun bombardment of skin with DNA-coated gold beads. The method of DNA inoculation (gene gun versus intramuscular injection) and the form of the DNA-expressed antigen (cell-associated versus secreted) determine whether T-cell response will be primarily type 1 or type 2. Gene gun-delivered DNA is known to initiate responses by transfected or antigen-bearing epidermal Langerhans cells that move lymph from bombarded skin to draining lymph nodes. Following intramuscular injections, the functional DNA appears to move as free DNA through blood to the spleen where antigen presenting cells initiate responses.

The nucleic acid vaccines of the present invention may be delivered either directly or indirectly. Direct delivery involves the direct introduction of nucleic acid vaccine into cells of the subject. For indirect delivery, the nucleic acid vaccine is first introduced into suitable cells by any method known in the art *in vitro*, then the cells containing the nucleic acid vaccine are administered to the subject. Vectors used with the nucleic acid vaccine of the present invention preferably contain strong mammalian promoters for high expression. Examples include attenuated *Salmonella spp.* and *Shigella spp.*

Direct delivery of the nucleic acid vaccines of the present invention can be accomplished by any of numerous methods known in the art. For example, the nucleic acid vaccines can be constructed as part of an appropriate nucleic acid expression vector and administered in a manner which introduces the nucleotide into cells of the subject, *e.g.*, by infection using a defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286); by direct injection of naked nucleic acid vaccine; by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont); by coating with lipids or cell-surface

receptors or transfecting agents; by encapsulation in liposomes, microparticles, or microcapsules; by administering the nucleic acid vaccine linked to a peptide which is known to enter the nucleus; and by administering the nucleic acid vaccine linked to a ligand subject to receptor-mediated endocytosis (see e.g., Wu and Wu, *J. Biol. Chem.* 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), and the like. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In a preferred embodiment, the nucleic acid vaccine is injected into the muscle of the subject to be immunized. The nucleic acid vaccines of the present invention can also be delivered as aerosols, and deliberate induction of injury to muscles prior to injection of DNA may be employed in some instances to enhance gene expression.

In a preferred aspect of the present invention, a nucleic acid vaccine or cellular vector vaccine is administered as a primer, and a chimeric polypeptide is subsequently administered as a booster. Alternatively, a chimeric polypeptide is administered as a primer, and a nucleic acid vaccine or cellular vector vaccine is subsequently administered as a booster.

Where the nucleic acid of the nucleic acid vaccine is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell, transformation can be carried out by any method known in the art. For example, such cells can be transformed using transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign nucleic acid into cells (see *e.g.*, Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92 (1985)) and may be used in accordance with the present invention. Usually, the method of transformation also includes the transfer of a selectable marker to the cells. Cells expressing the transferred gene can then be identified and isolated.

Cells into which a nucleic acid vaccine can be introduced for purposes of immunization encompass any desired, available cell type, and include but are not limited to epithelial

cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, *e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, and the like.

The resulting recombinant cells can be delivered to a subject by various methods known in the art. In a preferred embodiment, the recombinant cells are injected, *e.g.*, subcutaneously. In another embodiment, recombinant skin cells may be applied as a skin graft onto the patient. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are preferably administered intravenously. The cells can also be encapsulated in a suitable vehicle and then implanted in the subject (*see, e.g.*, Dionne et al. PCT Publication WO 92/19195, dated November 12, 1992). The amount of cells administered depends on a variety of factors known in the art, for example, the desired effect, subject state, rate of expression of the chimeric polypeptides, etc., and can readily be determined by one skilled in the art.

By way of example, and not by way of limitation, a nucleic acid vaccine according to the present invention may be generated as described by Lekutis et al. for an HIV nucleic acid vaccine (1997, *J. Immunol.* 158:4471-4477). Briefly, an expression vector is constructed with the promoter, enhancer and intron A of human cytomegalovirus (CMV) and the termination and polyadenylation sequences of bovine growth hormone in a plasmid backbone. Additionally, the nucleotide sequence for the signal sequence of tissue plasminogen activator is optionally substituted for the signal sequence of the antigen or the chemokine or another appropriate signal sequence. Alternatively, the nucleotide sequence for the signal sequence of tissue plasminogen activator is added onto the amino-terminus of the chimeric polypeptide. The resulting formulation is preferably injected intramuscularly.

Further examples of nucleic acid vaccines are set forth in Boyer et al. (1996, *J. Med. Primatol.*, 25:242-250), which describes the construction of a plasmid encoding the HIV-1 gp160 envelope glycopolypeptide as well as the *rev-tax* region cloned into pMAMneoBlue vector (Clontech, Inc., Palo Alto, CA), and a vector encoding the envelope glycopolypeptide and rev from HIV-1 strain MN under the control of the CMV promoter.

Another vector which can be used in the present invention is as described in Boyer et al. (1997, *Nature Medicine* 3:526-532).

For the practice of the present invention, the nucleotide sequence for the chimeric polypeptide(s) may be administered with a nucleotide sequence encoding additional chemokines (either the same chemokine(s) as the chemokine polypeptide component(s) of the chimeric polypeptide, and or different chemokine(s)) as well as functional equivalents thereof. Such nucleotide sequence(s) encoding additional chemokine(s) and/or functional equivalents of chemokine(s) can be incorporated into the same expression vector containing the nucleotide sequence encoding the chimeric polypeptide in such a manner that the chemokine is expressed. Alternatively, the nucleotide sequence encoding the chemokine fragments, chemokine derivatives, chemokine analogues, and/or chemokine truncation isoforms can be cloned into a separate expression vector (*e.g.*, as described above for the expression vector containing the sequences coding for the chimeric polypeptides). The expression vector which expresses the chimeric polypeptide(s) can be administered either simultaneously or sequentially with the expression vector that expresses the chemokine(s) and/or functional equivalents thereof. Alternatively, a mixture comprising the two expression vectors can then be administered to the subject.

5.3 Production of Nucleic Acids and Chimeric Polypeptides

Chimeric polypeptides of the present invention can be obtained by any method known in the art.

5.3.1 Production of Nucleic Acids

The individual chemokine and antigen nucleotide and amino acid sequences which comprise the chimeric polypeptides of the present invention are available to the public. These sequences are published in publicly available journal articles and in publicly accessible databases, many of which are available to the public via the Internet (e.g., see the Internet site of the National Center for Biotechnology Information: http://www.ncbi.nlm.nih.gov).

Any vertebrate cell potentially can serve as the nucleic acid source for the isolation of chemokine or antigen nucleic acids. The chemokine-encoding or antigen-encoding nucleic acid sequences can, for example, be isolated from vertebrate, mammalian, human, porcine, bovine, feline, avian, equine, canine, as well as additional primate sources, etc.

DNA encoding the any of the components (i.e., chemokine polypeptide(s), polypeptide linker(s) and antigen polypeptide(s)) of the chimeric polypeptides of the present invention may be obtained by standard procedures known in the art from cloned DNA (*e.g.*, a DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell (see, for example, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II.) Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences.

In the molecular cloning of the gene from cDNA, cDNA is generated from totally cellular RNA or mRNA by methods that are well known in the art. The gene may also be obtained from genomic DNA, where DNA fragments are generated (e.g. using restriction enzymes or by mechanical shearing), some of which will encode the desired gene. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

Once the DNA fragments are generated, identification of the specific DNA fragment containing all or a portion of the chemokine or antigen gene may be accomplished in a number of ways.

A preferred method for isolating a chemokine or antigen gene is by the polymerase chain reaction (PCR). PCR can be used to amplify the desired chemokine or antigen sequence in a genomic or cDNA library or from genomic DNA or cDNA that has not been incorporated into a library. Oligonucleotide primers which would hybridize to chemokine or antigen sequences can be used as primers in PCR.

Additionally, a portion of the chemokine or antigen gene or its specific RNA, or a fragment thereof, can be purified (or an oligonucleotide synthesized) and labeled, and the resulting generated DNA fragments may be screened by nucleic acid hybridization to the labeled probe (Benton, W. and Davis, R., 1977, Science 196:180; Grunstein, M. And Hogness, D., 1975, Proc. Natl. Acad. Sci. U.S.A. 72:3961). Those DNA fragments with substantial homology to the probe will hybridize. Chemokine or antigen nucleic acids can also be identified and isolated by expression cloning using, for example, anti-chemokine or anti-antigen antibodies for selection.

Alternatives to obtaining the chemokine or antigen DNA by cloning or amplification include, for example, chemically synthesizing the gene sequence from the known chemokine or antigen sequence or making cDNA to the mRNA which encodes the chemokine or antigen. Other methods are possible and within the scope of the invention.

Once a clone has been obtained, its identity can be confirmed by nucleic acid sequencing (by any method well known in the art) with further comparison to known chemokine or antigen sequences. DNA sequence analysis can be performed by a variety techniques known in the art. Such techniques include, for example, the method of Maxam and Gilbert (1980, Meth. Enzymol. 65:499-560), the Sanger dideoxy method (Sanger, F., et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74:5463), the use of T7 DNA polymerase (Tabor and Richardson, U.S. Patent No. 4,795,699), the use of an automated DNA sequenator (*e.g.*, Applied Biosystems, Foster City, CA) or the method described in PCT Publication WO 97/ 15690.

Nucleic acids which are hybridizable to a nucleic acid encoding a chemokine polypeptide, polypeptide linker or antigen polypeptide or to a functional equivalent of any of these polypeptides, can be isolated by nucleic acid hybridization under conditions of low, high, or moderate stringency (see also Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. USA 78:6789-6792).

5.3.2 Recombinant Production of Chimeric Polypeptides

Chemokine polypeptides, polypeptide linkers, antigen polypeptides and functional equivalents of such polypeptides can be obtained by any method known in the art

including, but not limited to, recombinant expression methods, purification from natural sources, and chemical synthesis.

For example, such polypeptides can be obtained by recombinant expression techniques. For obtaining a polypeptide by recombinant expression, the polypeptide gene or portion thereof is inserted into an appropriate cloning vector for expression in a particular host cell. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to phage vectors, plasmid vectors, phagemid vectors, phasmid vectors, cosmid vectors, virus vectors, and yeast vectors. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene). The vector system must be compatible with the host cell used.

The insertion into a cloning vector can be accomplished by a variety of techniques known to those of skill in the art. For example, insertion can be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. If the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and chimeric polypeptide(s) may be modified by homopolymeric tailing. The resulting recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In an alternative method, the desired gene may be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for the desired gene, for example, by size fractionation, can be performed prior to insertion into the cloning vector.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the chimeric polypeptide-encoding nucleic acid sequence enable generation of multiple copies of the chimeric polypeptide-encoding nucleic acid sequence.

Thus, the chimeric polypeptide-encoding nucleic acid sequence may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted chimeric polypeptide-encoding nucleic acid sequence from the isolated recombinant DNA.

The nucleotide sequence coding for a chimeric polypeptide(s) of the present invention can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted polypeptide-coding sequence. The necessary transcriptional and translational signals can also be supplied, for example, by the native chemokine and/or antigen gene and/or its flanking regions.

A variety of host-vector systems may be utilized to express the polypeptide-coding sequence. These systems include, without limitation, mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, adeno-associated virus, Pox-virus, retrovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and the polypeptide coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of a nucleic acid sequence encoding a chimeric polypeptide may be regulated so that the chimeric polypeptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a chimeric polypeptide of the present invention may be controlled by any promoter/enhancer element known in the art.

Expression of the chimeric polypeptide-encoding nucleic acid can be controlled by promoters, e.g., the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310); the promoter contained in the 3' long terminal repeat of Rous sarcoma

virus (Yamamoto, et al., 1980, Cell 22:787-797); the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445); the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731); or the *tac* promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25); see also "Useful polypeptides from recombinant bacteria" in Scientific American, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter; the ADC (alcohol dehydrogenase) promoter; PGK (phosphoglycerol kinase) promoter; alkaline phosphatase promoter; and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122); immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444); mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495); albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276); alpha-fetopolypeptide gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58); alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171); beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94); myelin basic polypeptide gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286); and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

A vector also can be used that comprises a promoter operably linked to a chimeric polypeptide-encoding nucleic acid, one or more origins of replication, and, optionally, one or more selectable markers (*e.g.*, an antibiotic resistance gene).

In a specific embodiment, an expression vector is made by subcloning a chimeric polypeptide coding sequence into the *Eco*RI restriction site of each of the three pGEX vectors (Glutathione S-Transferase expression vectors; Smith and Johnson, 1988, Gene 7:31-40). This approach permits expression of the chimeric polypeptide product from the subclone in the correct reading frame.

Expression vectors comprising the chimeric polypeptide-encoding nucleic acids can be identified by any of a variety of procedures known in the art, for example, nucleic acid hybridization, identification of marker gene functions, and expression of inserted sequences. In the nucleic acid hybridization approach, the presence of a chimeric polypeptide-encoding nucleic acid inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted chimeric polypeptide-encoding nucleic acid.

In the marker gene approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (*e.g.*, thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of a chimeric polypeptide-encoding nucleic acid in the vector. For example, if the chimeric polypeptide-encoding nucleic acid is inserted within the marker gene sequence of the vector, recombinants containing the chimeric polypeptide insert can be identified by the absence of the marker gene function.

Finally, recombinant expression vectors can be identified by assaying the chimeric polypeptide product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the chimeric polypeptide polypeptide in *in vitro* assay systems, *e.g.*, binding with of the chemokine, linker or antigen component to an antibody or binding of the chemokine component the chimeric polypeptide to a corresponding chemokine receptor.

Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product as desired. Expression of the chimeric polypeptide may be controlled, for example, by using an inducible promoter. Furthermore, host cells may be selected to effect various translational and post-translational processing and modifications (*e.g.*, glycosylation, phosphorylation of polypeptides). For example, expression in a bacterial system can be used to produce an unglycosylated core polypeptide product. Expression in yeast can be employed to produce a glycosylated product. Expression in mammalian cells can be used to ensure "native" glycosylation of a heterologous polypeptide. Furthermore, different vector/host expression systems may effect processing reactions to different extents.

The chimeric polypeptides of the present invention can be made by ligating, in the proper coding frame, the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, and expressing the chimeric product by methods commonly known in the art.

The chimeric polypeptides of the present invention may be isolated and purified by standard methods including chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of polypeptides. The functional properties may be evaluated using any suitable assay (see Section 5.5).

The nucleic acids of the present invention can be mutated as necessary *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions. Any technique for mutagenesis known in the art can be used, including, but not limited to, *in vitro* site-directed mutagenesis (Hutchinson et al., 1978, *J. Biol. Chem* 253:6551), use of TAB linkers (Pharmacia), mutation-containing PCR primers, *etc.*

The experimentation involved in mutagenesis consists primarily of site-directed mutagenesis followed by phenotypic testing of the altered gene product. Some of the more commonly employed site-directed mutagenesis protocols employ vectors that can provide single stranded as well as double stranded DNA, as needed. Generally, the mutagenesis protocol with such vectors is as follows. A mutagenic primer, *i.e.*, a primer

complementary to the sequence to be changed, but consisting of one or a small number of altered, added, or deleted bases, is synthesized. The primer is extended *in vitro* by a DNA polymerase. After several manipulations known in the art, the resulting double-stranded DNA is transfected into bacterial cells. The desired mutated DNA is then identified by methods well known in the art, and the desired polypeptide is purified from clones containing the mutated sequence. Protocols are known to one skilled in the art and kits for site-directed mutagenesis are widely available from biotechnology supply companies such as Amersham Life Science, Inc. (Arlington Heights, IL) and Stratagene Cloning Systems (La Jolla, CA).

In other specific embodiments, the chemokine derivative or analogue may be expressed as a fusion, or chimeric polypeptide product (comprising the polypeptide, fragment, analogue, or derivative joined via a peptide bond to a heterologous polypeptide sequence (of a different polypeptide)). Such a chimeric product can be made by ligating, in the proper coding frame, the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art and expressing the chimeric product by methods commonly known in the art.

5.3.3 Synthesis of Chimeric Polypeptides

In addition to the recombinant methods described above, the chimeric polypeptides (including the component chemokine, antigen and linker components) may be made by synthetic techniques, *e.g.*, by use of a peptide synthesizer, or other standard chemical methods known in the art (*e.g.*, see Hunkapiller, M., et al., 1984, Nature 310:105-111; Clark-Lewis et al., 1991, *Biochem.* 30:3128-3135 and Merrifield, 1963, *J. Amer. Chem. Soc.* 85:2149-2156. For example, the chimeric polypeptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (*e.g.*, *see* Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and Co., N.Y., pp. 50-60). The composition of the chimeric polypeptides may be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and Co., N.Y., pp. 34-49).

The chemokine polypeptides of the invention may be synthesized in their entirety by the sequential addition of amino acid residues or alternatively as fragment subcomponents which may be combined using techniques well known in the art, such as, for example, fragment condensation (Shin et al., 1992, *Biosci. Biotech. Biochem.* 56:404-408; Nyfeler et al., 1992, Peptides, Proc. 12th Amer. Pep. Soc., Smith and Rivier (eds), Leiden, pp 661-663; and Nokihara et al., 1990, Protein Research Foundation, Yanaihara (ed), Osaka, pp 315-320).

In a less preferred embodiment, chemokine or antigen derivatives can be obtained by proteolysis of the chemokine or antigen polypeptide followed by purification using standard methods such as those described above (*e.g.*, immunoaffinity purification).

In another alternate embodiment, native chemokine or antigen polypeptides can be purified from natural sources using standard methods such as those described above (*e.g.*, immunoaffinity purification).

5.4 Formulations

The formulations of the invention generally comprise, in association with a pharmaceutically acceptable carrier or excipient, (1) a chimeric polypeptide and/or (2) a nucleic acid encoding a chimeric polypeptide. The nucleic acid encoding a chimeric polypeptide may encode one or more chimeric polypeptides and has the capacity to express the nucleotide sequence encoding the chimeric polypeptide.

Pharmaceutically acceptable components of the formulations (e.g., carriers and excipients) are well known in the art. For example, these components include physiological saline, buffered saline, dextrose, water, glycerol, ethanol, sterile isotonic aqueous buffer, and combinations thereof. One example of an acceptable carrier is a physiologically balanced culture medium containing one or more stabilizing agents such as stabilized, hydrolyzed polypeptides, lactose, and the like. The carrier is preferably sterile. The formulation should suit the mode of administration.

In addition, if desired, the vaccine or composition preparation may also include minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering

agents, and /or adjuvants which enhance the effectiveness of the vaccine or composition. Suitable adjuvants may include, but are not limited to: mineral gels, *e.g.*, aluminum hydroxide; surface active substances such as lysolecithin, pluronic polyols; polyanions; peptides; oil emulsions; alum, MDP, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine, and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine. The effectiveness of an adjuvant may be determined by comparing the induction of antibodies directed against a chimeric polypeptide of the present invention composition in the presence and in the absence of various adjuvants.

The composition can be a liquid solution, suspension, emulsion, microemulsion, tablet, pill, capsule, sustained release formulation, or powder. Oral formulation can include standard excipients such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like.

The chimeric polypeptides of the present invention may be formulated as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids, such as, for example, hydrochloric or phosphoric acids, or organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with free carboxyl groups may also be derived from inorganic bases, e.g., sodium potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

The chimeric polypeptides may be constructed as univalent, divalent or multivalent. The chimeric polypeptides may contain multiple copies of the same antigen species or multiple (i.e., two or more) species of antigen.

The chimeric polypeptides are administered to the subject in an amount sufficient to elicit an enhanced immune response, enhance an immune response and/or cause an immunizingly effective response. The precise dose of the composition to be employed in the formulation will depend on a variety of factors known to those of skill in the art. For example, factors to be considered include the route of administration and the nature of the subject to be immunized. The effect of such factors, and other factors known in the art, is

readily determined by one of skill in the art according to standard clinical techniques. Effective doses of the chimeric polypeptides or compositions of the present invention may also be extrapolated from dose-response curves derived from animal model test systems.

The invention also provides a pharmaceutical pack or kit comprising one or more containers comprising one or more of the chimeric polypeptides of the invention and/or formulations of the present invention. A notice can be associated with such container(s). The notice is preferably in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products. Further, the notice may reflect approval by the agency for manufacture, use or sale for human administration.

Generally, the ingredients are supplied either separately or mixed together in unit dosage form. An example of the former is a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachette indicating the quantity of active agent. Where the composition is administered by injection, an ampoule of sterile diluent can be provided so that the ingredients may be mixed prior to administration.

In a specific embodiment, a lyophilized chimeric polypeptide of the invention is provided in a first container, and a second container comprises diluent consisting of an aqueous solution of 50% glycerin, 0.25% phenol, and an antiseptic (*e.g.*, 0.005% brilliant green).

Many methods may be used to administer the compositions and formulations of the invention. These include but are not limited to: oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intraosseous, intranasal routes, and via scarification (scratching through the top layers of skin, *e.g.*, using a bifurcated needle). The nucleic acid vaccines or the invention can be administered by any method known in the art for delivery of DNA to subject (for example, as described in Section 5.3 supra).

5.5 Assays

The activity of the chimeric polypeptides of the present invention can be validated by monitoring the immune response in test animals following immunization with a composition containing a chimeric polypeptide. The response of such test animals can be

compared to a response in control animals immunized with a corresponding antigen alone and control animals administered with a corresponding chemokine and a corresponding antigen, wherein the chemokine and antigen are not connected by a polypeptide linker. Other controls will be apparent to persons of skill in the art.

An immune response is indicated, for example, by generation of a humoral (antibody) response and/or cell-mediated immunity. Test animals may include mice, hamsters, dogs, cats, monkeys, rabbits, chimpanzees, etc., and eventually human subjects. Assays for humoral and cell-mediated immunity are well known in the art. The immune response of the test subjects can be analyzed by various approaches well known in the art such as, but not limited to, testing the reactivity of the resultant immune serum to the antigen of the chimeric polypeptide, as assayed by known techniques (*e.g.*, immunosorbant assay (ELISA), immunoblots, radioimmunoprecipitations, and the like).

Methods of introducing the composition into assays of the present invention may include oral, intracerebral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intraosseous, intranasal or any other standard routes of immunization.

As one example of suitable animal testing, a chimeric polypeptide of the present invention may be tested in mice for the ability to enhance an antibody response to an antigen component of the chimeric polypeptide and/or the delayed-type hypersensitivity (DTH) response, measured by an increase in footpad swelling after inoculation in the footpad of the test animal. These measurements can then be compared to corresponding measurements in control animals, e.g., animals administered antigen alone or chemokine alone, or antigen and chemokine administered in a mixture, or an antigen-chemokine molecule without a polypeptide linker. As an example, BALB/c mice may be used as test animals. The members of the test group are each administered a fixed amount of chimeric polypeptide.

Serum samples may be drawn from the mice after the final inoculation (for example every one or two weeks after inoculation). Serum can be analyzed for antibodies against the antigen using known methods in the art, *e.g.*, using an ELISA. DTH responses to the antigen may be measured after the final inoculation (e.g. within 1-7 days). An increase in the serum titer of antibodies recognizing the antigen and/or an increase in footpad

swelling in the animals receiving the chimeric polypeptides as compared to the serum titer of the control animals, indicates that the chimeric polypeptide enhances the immune response to antigen.

6. EXAMPLES

The following examples are illustrative of the practice of the invention and are not intended to limit the scope of the invention.

6.1 Examples 1 to 4: Synthesis of Chimeric Polypeptides

Examples 1 to 4 (see below) show chimeric polypeptides that were prepared by the present inventors using standard recombinant DNA techniques.

Example I

*MKISAAALTIILTAAALCAP***ASPYGSDTTPCCFAYLSLELPRAHVKEYFYTSSKCSNLAVV**
FVTRRNRQVCANPEKKWVQEYINYLEMSEPRVPITQNPCPPPIVQNIQGQMVHQAISPR
TLNAWVKVVEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKETINEEA
AEWDRVHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILG
LNKIVRMYSPTSILDIRQGPKEPFRDYVDRFYKTLRAEQASQEVKNWMTETLLVQNANPD
CKTILKALGPAATLEEMMTACQGVGGPGHKARVLAEAMSQVTNSATIMMQRGNFRNQR
KIVKCFNCGKEGHTARNCRAPRKKGCWKCGKEGHQMKDCTERQANFLGKIWPSYKGR
PGNFLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDPSSQ

Example 2

*MRLSTATLLLLLASCL***SPGHGILEAHYTNLKCRCSGVISTVVGLNIIDRIQVTPPGNGCPKT**
EVVIWTKMKKVICVNPRAKWLQRLLRHVQSKSLSSTPQAPVSKRRAAEPRVPITQNPC
PPPIVQNIQGQMVHQAISPRTLNAWVKVVEEKAFSPEVIPMFSALSEGATPQDLNTMLNT
VGGHQAAMQMLKETINEEAAEWDRVHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIG
WMTNNPPIPVGEIYKRWIILGLNKIVRMYSPTSILDIRQGPKEPFRDYVDRFYKTLRAEQA
SQEVKNWMTETLLVQNANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLAEAM
SQVTNSATIMMQRGNFRNQRKIVKCFNCGKEGHTARNCRAPRKKGCWKCGKEGHQMK

DCTERQANFLGKIWPSYKGRPGNFLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKEL
YPLTSLRSLFGNDPSSQ

Example 3

*MATLRVPLLVALVLLAVAIQTSDA***GPYGANVEDSICCQDYIRHPLPSRLVKEFFWTSKSC**
RKPGVVLITVKNRDICADPRQVWVKKLLHKLSEPRVPITQNPCPPPIVQNIQGQMVHQAI
SPRTLNAWVKVVEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKETIN
EEAAEWDRVHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRW
IILGLNKIVRMYSPTSILDIRQGPKEPFRDYVDRFYKTLRAEQASQEVKNWMTETLLVQNA
NPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLAEAMSQVTNSATIMMQRGNFR
NQRKIVKCFNCGKEGHTARNCRAPRKKGCWKCGKEGHQMKDCTERQANFLGKIWPSY
KGRPGNFLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDPSSQ

Example 4

*MQVPVMLLGLLFTVAGWSIHVLA***QPDAVNAPLTCCYSFTSKMIPMSRLESYKRITSSRCP**
KEAVVFVTKLKREVCADPKKEWVQTYIKNLDRNQMRSEPTTLFKTASALRSSAPLNVK
LTRKSEANASTTFSTTTSSTSVGVTSVTVNEPRVPITQNPCPPPIVQNIQGQMVHQAISP
RTLNAWVKVVEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKETINEE
AAEWDRVHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIIL
GLNKIVRMYSPTSILDIRQGPKEPFRDYVDRFYKTLRAEQASQEVKNWMTETLLVQNANP
DCKTILKALGPAATLEEMMTACQGVGGPGHKARVLAEAMSQVTNSATIMMQRGNFRNQ
RKIVKCFNCGKEGHTARNCRAPRKKGCWKCGKEGHQMKDCTERQANFLGKIWPSYKG
RPGNFLQSRPEPTAPPEESFRSGVETTTPPQKQEPIDKELYPLTSLRSLFGNDPSSQ

The italicized polypeptide regions correspond to a signal peptide sequence for secretion of the mature polypeptide. The signal peptide sequence is not retained by the secreted polypeptide. In different expression systems, the site of cleavage from the signal peptide may vary ± two amino-acid residues.

The bold text represents the chemokine portion of the molecule, corresponding to: murine RANTES (Example 1) (an alternative signal peptide has been reported for murine

RANTES, *MKISAAALTIILTAAALCTPAP*), murine BLC (Example 2), murine MDC (Example 3), murine MCP-1/JE (Example 4).

The underlined region is the hinge region of IgG 2a/b, as reported for mouse strain C57BL/6. In these examples, the antibody hinge region was derived from mouse genes, to be used in animal studies; however, for human use, human hinge regions are preferable.

The remaining part of the polypeptide sequence corresponds to the p24 core antigen of HIV-1, strain IIIB.

6.1.1 Primers Used in Preparation of Examples 1 to 4

Cloning Murine RANTES cDNA of Example 1

Forward Primer: MRANFOR: 5=-CCGGATCCGCGGGTACCATGAAGATCTC-3=

(contains a BamHI site followed by nucleotides B9 to 11 from translation start site)

Reverse Primer: MRANREV: 5=-GGGAATTCAAGAAACCCTCTATCCTAGCT-3=

(contains a EcoRI site followed by nucleotides 291 to 271 from translation start site)

Generating RANTES-p24 of Example 1

The Primers below comprise the last 15 nucleotides from the mouse RANTES cDNA stop codon, the hinge sequence from the mouse IgG2 heavy chain, followed by the first 15 nucleotides from the translational start site of the HIV-1 p24 cDNA.

Forward Primer: MRHP24FOR: 5=-TATTTGGAGATGAGCGAGCCCAGAGTGCCCATAA CACA GAACCCCTGTCCTCCACCTATAGTGCAGAAC-3=

Reverse Primer: MRHP24REV: 5=-GTTCTGCACTATAGGTGGAGGACAGGGGTTCTGT GTTA TGGGCACTCTGGGCTCGCTCATCTCCAAATA-3=

P24REV: 5=-GGCTCTAGATTATTGTGACGAGGGGTCGTT-3=

(nucleotides 291 to 271 from translation start site)

Cloning Murine BLC cDNA of Example 2

Forward Primer: MBLCFOR2: 5=-GGGGATCCAGAATGAGGCTCAGCACAGC-3=

(contains a BamHI site followed by nucleotides B3 to 17 from translation start site)

Reverse Primer: MBLCREV2: 5=-GGGTCTAGATCAGGCAGCTCTTCTCTTAC-3=

(contains a XbaI site followed by nucleotides 362 to 343 from translation start site)

Generating BLC-p24 of Example 2

The Primers below comprise the last 15 nucleotides from mouse BLC cDNA stop codon, the hinge sequence from the mouse IgG2 heavy chain, followed by the first 15 nucleotides from the translational start site of the HIV-1 p24 cDNA.

Forward Primer: MBHP24FOR: 5=-AAGAGAAGAGCTGCCGAGCCCAGAGTGCCCATAA CAC AGAACCCCTGTCCTCCACCTATAGTGCAGAAC-3=

Reverse Primer: MBHP24REV: 5=-GTTCTGCACTATAGGTGGAGGACAGGGGTTCTGT GTT ATGGGCACTCTGGGCTCGGCAGCTCTTCTCTT-3=

Cloning Murine MDC cDNA of Example 3

Forward Primer: MuMDCF: 5= CCGGATCCAC ACCATGGCTA CCCTGCGT -3=

(contains a BamHI site)

Reverse Primer: MuMDCR: 5= GGGAATTCCT CCCTAGGACA GTTTATGG -3=

(contains a EcoRI site)

Generating Murine MDC-p24 of Example 3

The Primers below comprise the last 15 nucleotides from mouse MDC stop codon, the hinge sequence from the mouse IgG2 heavy chain followed by the first 15 nucleotides from the translational start site of the HIV-1 p24 cDNA.

Forward Primer: Example 3F: 5'- CTCCATAAAC TGTCCGAGCC CAGAGTGCCC ATAA CACAGA ACCCCTGTCC TCCACCTATA GTGCAGAAC-3'

Reverse Primer: Example 3R: 5= GTTCTGCACT ATAGGTGGAG GACAGGGGTT CTG TGTTATG GGCACTCTGG GCTCGGACAG TTTATGGAG-3=

Cloning Murine MCP-1 cDNA of Example 4

Forward Primer: MuMCP1F: 5= CCGGATCCTC CACCACCATG CAGGTCCC-3=

(contains a BamHI site)

Reverse Primer: MuMCP1R: 5= GGGAATTCCA CACTAGTTCA CTGTCACA-3=

(contains an EcoRI site)

Generating Murine MCP-1-p24 of Example 4

The Primers below comprise the last 15 nucleotides from mouse MCP-1/JE stop codon, the hinge sequence from the mouse IgG2 heavy chain followed by the first 15 nucleotides from the translational start site of the HIV-1 p24 cDNA.

ForwardPrimer: Example 4F: 5=-TGACAGTGAACGAGCCCAGAGTGCCCATAACACAGA ACCCCTGTCC TCCACCTATA GTGCAGAAC-3=

Reverse Primer: Example 4R: 5= GTTCTGCACT ATAGGTGGAG GACAGGGGTT CTGT GTT ATG GGCACTCTGG GCTCGTTCAC TGTCACACT-3=

Preparation of total cellular mouse RNA: Total cellular RNA was obtained from the spleen or muscle dissected from a 6-8 weeks old female C57/BL mouse using Trizol from Gibco BRL/Life Technologies (Gaithersburg, MD, USA) according to the manufacturer=s instructions. Briefly, 50-100 mg of the spleen or muscle tissue was homogenized in 1.4 ml Trizol by passing several times through an 18-gauge needle. After homogenization, the mixture was vortexed and allowed to stand at Room Temperature (RT) for 10 min. Next, 300 μl of chloroform was added, the mixture vortexed again and allowed to stand at RT for another 5 min. The tube was spun at 12,000 rpm for 20 min at 4°C. The upper phase was transferred to a new tube and mixed with an equal volume of isopropanol. The tube was allowed to stand at RT for 10 min and spun at 12,000 rpm for 10 min at 4°C. The supernatant was discarded, and the pellet washed with 70% ethanol. After air-drying for 5 min the pellet was re-dissolved in 50 μl of sterile DEPC-treated water.

Generation of murine chemokine expression constructs: First strand cDNA synthesis was carried out using Superscript II Rnase H⁻ reverse transcriptase from Gibco BRL/Life Technologies, (Gaithersburg, MD, USA). 2 μg of total cellular RNA from mouse spleen was added with 250 ng random hexamers and mixed with sterile water to total 12 ml of solution. The mixture was incubated at 25EC for 10 min and then chilled on ice. The tube was added with 4 ml of 5X first strand buffer, 2 ml 0.1 M DTT and 1 ml 10 mM dNTP mix and incubated at 42EC for 2 min. 200U of Superscript II was added and the tube re-incubated at 42EC for 50 min, and then heated at 70EC for 15 min to inactivate the enzyme. PCR amplification was performed with 2 μl of the RT mix in a 50-μl final volume using the Expand High Fidelity PCR kit from Boehringer Mannheim (Germany) and the Trio-Thermoblock from Biometra (Biometra GmbH, Gottingen, Denmark). Each reaction contained 200 mM dNTPs, 1.5 mM $MgCl_2$ and 100 ng each of the forward and reverse primers. 30 PCR cycles were carried out as follows: 94EC for 1 min, 62EC for 1 min and 68°C for 1 min. The mouse RANTES PCR product was amplified with the primers MRANFOR and MRANREV and digested with BamHI and EcoRI. The mouse BLC PCR product was amplified with primers MBLCFOR2 and MBLCREV2 and re-digested with BamHI and XbaI. Both cDNAs were cloned into the expression vector pCDNA3.1(+) from Invitrogen (Carlsbad, CA, USA) to generate the murine chemokine expression constructs pCMV-mRANTES and pCMV-mBLC respectively.

Similarly, to amplify murine MCP-1 and murine MDC, primers indicated above (50 μM) were used. Templates were 1 μg of cDNA obtained from the I.M.A.G.E. consortium. Clone #AA620145 was used to amplify murine MCP-1 and #175762 was used to amplify murine MDC. PCR condition used were 94EC for 2 min, then 5 cycles at 94EC for 1min, 50EC for 1 min 94EC for 1 min, followed by 25 cycles at 94EC for 1 min, 60EC for 1 min 94EC for 1 min, and a final extension at 72E for 10 min. Total volume of PCR was 100 μl. Both cDNAs were cloned into the expression vector pCDNA3.1(+) from Invitrogen (Carlsbad, CA, USA) to generate the murine chemokine expression constructs pCDNAMuMCP-1 and pCDNA-MuMDC, respectively.

Generation of expression constructs for chimeric molecules: The 5= fusions of mouse chemokines RANTES and BLC to the HIV-1 p24 cDNA were carried out using a 2-step Amega-primer≅ method for site-directed mutagenesis. To generate the Example 1 molecule, a first PCR reaction was set up to re-amplify the mouse RANTES cDNA using the forward primer, MRANFOR and the reverse primer, MRHP24REV and 50 ng of pCMV-mRANTES as template. 30 cycles of PCR reaction were carried out as follows: 94EC for 1 min, 42EC for 1 min, and 68EC for 1 min. A second PCR reaction was carried out using the primers MRHP24FOR and P24REV with 50 ng of pCMV-P24 as template. 30 cycles of PCR were performed as follows: 94EC for 1 min, 44EC for 1 min, and 68EC for 2 min. 50 ng of each of the two resultant PCR products were used in a third PCR reaction with the forward primer MRANFOR and the reverse primer P24REV at the following conditions: 94EC for 1 min, 62EC for 1 min, and 68EC for 2 min. The resultant PCR product was digested with BamHI and XbaI and cloned into pcDNA3.1 (+).

To generate the Example 2 molecule, a first PCR reaction was set up to re-amplify the mouse BLC cDNA using the forward primer, MBLCFOR2 and the reverse primer, MBHP24REV and 50 ng of pCMV-mBLC as template. 30 cycles of PCR reaction were carried out as follows: 94EC for 1 min, 46EC for 1 min, and 68EC for 1 min. A second PCR reaction was carried out using the primers MBHP24FOR and P24REV with 50 ng of pCMV-P24 as template. 30 cycles of PCR were performed as follows: 94EC for 1 min, 44EC for 1 min, and 68EC for 2 min. 50 ng of each of the two resultant PCR products were used in a third PCR reaction with the forward primer MBLCFOR2 and the reverse primer P24REV at the following conditions: 94EC for 1 min, 62EC for 1 min, and 68EC for

2 min. The resultant PCR product was digested with BamHI and XbaI and cloned into pcDNA3.1 (+).

To generate the Example 3 and the Example 4 molecules, analogous methods were used.

To generate the Example 3 molecule, a first PCR reaction was set up to amplify the mouse MDC-1 DNA attached to the mouse IgG hinge region and the first 5 amino-acids of p24 using the forward primer, MuMDCF and the reverse primer, Example 4R (both 10 μM) and 1 μg of the I.M.A.G.E. consortium Clone #17562 as template. 30 cycles of PCR reaction were carried out as follows: 94EC for 2=, then 5 cycles at 94EC for 1 min, 50EC for 1 min, 72EC for 1 min, followed by 25 cycles at 94EC for 1, 60EC for 1 min, 72EC for 1 min, and a final extension at 72E for 10 min. A second PCR reaction was carried out using the primers Example 4F, and P24REV (10 μM) with 1 μg of pCMV-P24 as template. 30 cycles of PCR were performed as follows: 94EC for 2 min, then 5 cycles at 94EC for 1 min, 50EC for 1 min, 72EC for 1 min, followed by 25 cycles at 94EC for 1 min, 60EC for 1 min, 72EC for 1 min, and a final extension at 72E for 10 min. 8 μg of each of the two resultant PCR products were used in a third PCR reaction with the forward primer MuMDCF and the reverse primer P24REV at the following conditions: 94EC for 2 min, then 5 cycles at 94EC for 1 min, 50EC for 1 min, 72EC for 1 min, followed by 25 cycles at 94EC for 1 min, 60EC for 1 min 72EC for 1 min, and a final extension at 72E for 10 min. The resulting PCR product was digested with BamHI and XbaI and cloned into pcDNA3.1 (+).

To generate the Example 4 molecule, a first PCR reaction was set up to amplify the mouse MCP-1 DNA attached to the mouse IgG hinge region and the first 15 nucleotides of p24 using the forward primer, MuMCP1F and the reverse primer, Example 4R and 1 μg of the I.M.A.G.E. consortium Clone #AA620145 as template. PCR reaction were carried out as follows: 94EC for 2 min, then 5 cycles at 94EC for 1 min, 50EC for 1 min, 72EC for 1 min, followed by 25 cycles at 94EC for 1 min, 60EC for 1 min, 72EC for 1 min, and a final extension at 72E for 10 min. A second PCR reaction was carried out using the primers Example 4F and P24REV with 1 μg of pCMV-P24 as template. 30 cycles of PCR were performed as follows: 94EC for 2 min, then 5 cycles at 94EC for 1 min, 50EC for 1 min, 72EC for 1 min, followed by 25 cycles at 94EC for 1 min, 60EC for 1 min, 94EC for 1

min, and a final extension at 72E for 10 min. 8 μg of each of the two resultant PCR products were used in a third PCR reaction with the forward primer MuMCPF and the reverse primer P24REV at the following conditions: 94EC for 2 min, then 5 cycles at 94EC for 1 min, 50EC for 1 min, 72EC for 1 min, followed by 25 cycles at 94EC for 1 min, 60EC for 1 min, 72EC for 1 min, and a final extension at 72E C for 10 min. The resulting PCR product was digested with BamHI and XbaI and cloned into pcDNA3.1 (+).

DNA sequencing: DNA sequencing of constructs pCMV-mRantes and pCMV-mBLC (Examples 1 and 2) was carried out in the sequencing facility at the Institute of Molecular and Cell Biology (IMCB). DNA sequencing of constructs pCMV-MuMDC and pCMV-MuMCP-1 (Examples 3 and 4) was carried out in the Biopolymer Sequencing Facility at the University of Maryland at Baltimore (UMB). 200 ng of the double-stranded templates and 10 ng of the primer were used for the dideoxy method with the Taq DyeDeoxy terminator cycle sequencing kit and the automated DNA sequencer 373A (IMCB) or 377 (UMB) from PE Applied Biosystems (Foster City, CA, USA).

Preparation of plasmid DNA: Plasmids for injection were prepared using the ENDOFREE™ kit, available from Qiagen Corporation, and the resulting plasmid preparations contained less than 0.05 ng/mg DNA, as determined by the limulus amoebocyte lysate assay from BioWhittaker, Inc. (Walkersville, MD, USA). The plasmid preparations were stored at 4°C in normal saline until injected.

Cell lines: The human epitheloid cervical carcinoma cell line HeLa and the human embryonic kidney cell line, 293, bearing the large T antigen from SV40 (293T) were purchased from American Type Cell Collection (ATCC, Manassas, VA, USA). The cells were cultured in EMEM containing 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, and 10% fetal bovine serum and were maintained at 37°C in 5% CO_2. Me10 is a murine fibroblast cell line, obtained from ATCC. 10ME Cells were were cultured in DMEM containing 2 mM L-glutamine, and 10% fetal bovine serum and maintained at 37°C in 5% CO_2.

Cell transfections: Transfection experiments were performed using Superfect™ transfection reagent from Qiagen (Valencia, CA, USA). The day before the transfection, 1.5×10^5 HeLa or 293T cells were seeded into 6-well tissue culture plates in 2.5 ml

complete media. The cells were 40-60% confluent on the day of transfection. For the transfection, 150 μl of serum free EMEM containing 5 μg of plasmid DNA was mixed well with 75 μg Superfect™ transfection reagent and allowed to stand at RT for 10 min to allow complex formation. Growth medium was aspirated from the cells and the cells washed once with 4 ml PBS. The DNA-Superfect™ mixture was diluted with 1 ml of complete growth medium and added to the cells. They were then re-incubated at 37EC and 5% CO_2 for 3 h, after which the medium was aspirated and the cells were washed again with 4 ml PBS. 2.5 ml fresh complete medium was added to the cells and incubation at 37EC and 5% CO_2 was continued for another 48-72 h after which the cell media was harvested.

Expression of recombinant polypeptides in baculovirus: Expression of Recombinant polypeptides in Baculovirus infected cells can be pursued by using standard protocols (for example, as described in current Protocols in Molecular Biology).

p24 ELISA assay: p24 ELISA assays were performed using a p24 ELISA kit from R&D Systems Inc. (Minneapolis, MN, USA). Briefly, 200 μl of cell media harvested from transfected cells were added to each well in an assay strip, pre-coated with anti-p24 antibodies. The strips were incubated at 37EC for 2 h. The wells were washed 4 times with wash buffer (PBS with 0.5% Tween) and added with 100 μl detector antibody each. They were re-incubated at 37EC for 1 h and washed again as before. 100 μl of the 1000-fold diluted streptavidin-conjugated horseradish peroxidase was added to each well and incubation carried out at RT for 30 min. The wells were washed again 4 times. 100 μl of TMB color reagent was added to each well and incubation carried out at RT for 17 min, after which 50 μl stop solution was added. Absorbance was read at 450 nm within 15 min of stopping reaction. In all experiments, standards were simultaneously assayed over a range from 0.006125 to 0.04 ng/ml. Results were as follows:

Table 3

p24 ELISA of culture media harvested from transfected cells (2/8/99)

		2-fold dilution (ng/ml)		Final value (ng/ml)	
Cell line	Construct	Day 5	Day 7	Day 5	Day 7
293T	pcDNA3.1(+)	0	0	0	0
	pRANP24	2.031	2.154	4.062	4.308
	Example 1	0.802	1.459	1.604	2.918
	Example 2	1.839	2.021	3.678	2.918

	Example 3	2.159	2.097	4.318	4.194
	Example 4	1.952	2.182	3.904	4.364
10ME	pcDNA3.1(+)	0.002	0	0.004	0
	pRANP24	2.054	1.673	4.108	3.346
	Example 1	0.161	0.241	0.322	0.482
	Example 2	0.019	0.019	0.038	0.038
	Example 3	0.175	0.114	0.35	0.228
	Example 4	0.179	0.165	0.358	0.33
NIH3T3	pcDNA3.1(+)	0.002	0.076	0.004	0.152
	pRANP24	1.874	1.681	3.748	3.362
	Example 1	0.136	0.189	0.272	0.378
	Example 2	0.074	0.08	0.152	0.16
	Example 3	0.201	0.246	0.402	0.492
	Example 4	0.929	1.371	1.858	2.742

Notes:
(1) $1X10^5$ cells were transfected with 5 μg of plasmid DNA with Superfect (Qiagen)
(2) Cell culture were harvested at specified days following transfection and analysed for secreted p24 proteins
(3) p24 ELISA kit from R&D (IHV core facility)

Western Blot: Recombinant Example 1, Example 2, Example 3 and Example 4 molecules secreted into the culture media of baculovirus-infected cells polypeptide were loaded on a 10% SDS-PAGE gel. The gel was run at 25 mA for 45 min. The polypeptides were transferred onto Hybond C-extra membranes (Amersham) in 1X Towbin buffer (20% methanol, 192 mM glycine and 25 mM Tris base) at 200 mA for I h in 4°C. The membranes were pre-incubated with blocking buffer (PBS containing 5% skim milk) at RT for 1-2 hrs. They were washed twice in wash buffer (PBS containing 0.05% Tween-20) at RT for 5 min. Primary antibody anti-HIV-1 p24 antibody from NEN (Boston, MA, USA) was added to the membrane and incubation was carried out at RT for 1 h. The membranes were washed twice at RT for 15 min with wash buffer. Secondary antibody containing conjugated horseradish peroxidase (Sigma, St. Louis, MO) was added and incubation was carried out for 30 min at RT. The membranes were washed as before and 1:1 ECL reagents (Pierce, Rockford, IL, USA) was added. Incubation was carried out for 5 min, after which the membranes were exposed to X-ray film for 2-10 min before the films were developed.

6.2 Examples 5: Immunizations

B10.D2 and Balb/c female mice aged 6-8 weeks were used for the experiments. Mice were maintained in specific pathogen-free conditions. Mice were separately injected with naked DNA or recombinant polypeptides expressed in baculovirus cell lines. The experiments were conducted according to institutional guidelines.

Injection of recombinant polypeptides: Groups of four mice were injected with saline alone, 20 μg of recombinant HIV-1 p24 antigen and 20μg of bovine serum albumin, 20μg of recombinant p24 and 20μg each of recombinant mouse chemokine (RANTES, BLC, MDC, or MCP-1), or 20μg each of recombinant chimeric molecules (Example 1, Example 2, Example 3 or Example 4). 20μg of each purified polypeptide concentrated from the supernatant of the baculovirus cell line was injected intramuscularly, by either needle injection or jet injection in the tibialis anterior. Experiments were performed with and without Freund=s adjuvant. Boosting injections were performed every two weeks for 8 weeks.

Injection of a combination of naked plasmid DNA and recombinant polypeptides: Groups of four mice can be injected with plasmid DNA at day 0 and boosted with recombinant polypeptides at day 14. Alternatively, mice can also be injected with recombinant polypeptides at day 0 and boosted with plasmid DNA at day 14. A sub-population of the mice responded to the chimeric polypeptides, and a subpopulation of the control group receiving pRANP24 responded positively. Data for the responders is set forth below:

Table 4

Group	DNA construct	Mouse#	Day 0	Wk 2	Wk 4	Wk 6	Wk 8	Wk 10
1	pRANP24	1	30.08	1555.54*	1356.8*	758.4	877.52	276.98
		2	27.28	23.92	836.16	745.28	1022.3	165.4
2	Example 1	1	54.56	24.77	1507.2*	1403.31*	1605.1	944.01
3	Example 3	1	28.05	13.92	865.6	3244.52*	3144.46**	1446.78
4	Example 4	1	21.6	7.52	83.44	876.16	1500	719.56
		2	38.99	29.44	1106.88	1642.56*	2884**	457.11

Notes:
(1) 200 μg plasmid DNA injected i.m. at day 0, wk2 and wk4 in 6-8 wk old female Balb/c mice
(2) Results are the average of two readings (μg/ml of total anti-p24 antibody)

(3) Lowest dilution of sera analysed in ELISA for day 0 to wk 6 =320X
(4) Lowest dilution of sera analysed in ELISA for wk 8 = 640X
(5) * denotes value out of std range at 320X dilution of sera sample
(6) ** denotes value out of std range at 640X dilution of sera sample

These data indicate that, whereas the response to the control (pRANP24) tends to decrease with time, the response to the chimeric polypeptides of the present invention is consistently maintained for a longer period.

Injection of naked plasmid DNA: In a similar experiment, groups of mice can be injected with either the vector DNA, pcDNA3.1(+) alone, equal amounts of pCMV-P24 and pcDNA3.1(+), equal amounts of pCMV-P24 and one of the four mouse chemokine expression constructs (pCMV-mRANTES, pCMV-mBLC, pCMV-MuMDC, or pCMV-MuMCP-1) or one of the four expression constructs for the chimeric molecules (Example 1, Example 2, Example 3 or Example 4). Mice can receive plasmid DNA intramuscularly in either quadriceps, or intradermally at the base of the tail on day 0 and 14 of each experiment. Plasmid DNA can also be introduced intramuscularly into separate groups of mice by jet injection at the same time-points of the experiments.

Expression of recombinant HIV-1 p24 polypeptide: To produce recombinant HIV-p24 polypeptide in bacteria, pCMV-P24 was cloned into the bacteria expression vector, pGEX-4T-1 (Pharmacia) and expressed as a GST-fusion polypeptide. Briefly, pCMV-P24 was first digested with XbaI, and the ends filled-in with dNTPs and Klenow (NEN). The construct was first digested with NotI and the ends filled-in and was further digested with pGEX-4T-1. The construct was then digested with XhoI and then ligated with the p24 cDNA. The resultant plasmid, pGEX-4T-1-P24 was transformed into the bacteria strain BL21 and selected in 50 μg/ml ampicillin. To express recombinant p24 polypeptide, transformed cells were grown overnight at 37°C in 10 ml 2XTY media supplemented with 50 μg/ml ampicillin. The next morning, 5 ml of cell culture was inoculated into 500 ml 2X TY supplemented with 150 μg/ml ampicillin and grown at 30°C till OD_{600} reached 0.7-1.0. The cells were induced with 0.1 mM isopropyl-1-thio-b-D-galactopyranoside (IPTG) at 25°C for 18 h. Cells were resuspended in Tris/NaCl (50mM Tris-HCl, pH 7.5, 150 mM NaCl) containing 1 mM EDTA, 1 mM dithiothreitol (DTT), and 0.5% Triton-X 100 and were further treated with 0.1 mg/ml lysozyme for 1 h at 4°C. Cells were sonicated on ice and the supernatant was obtained by ultracentrifugation at 16 000 rpm for 30 min at 4°C. The supernatant was incubated with glutathione-sepharose 4B beads (Pharmacia) at 4°C for 1

h. The beads were washed several times with Tris/NaCl (50mM Tris-HCl, pH 7.5, 150 mM NaCl) containing 1 mM EDTA and 0.5% Triton-X 100 followed by cleavage with thrombin (Sigma) for 2 h in 50 mM Tris HCl (pH 7.5), 150 mM NaCl and 2.5 mM $CaCl_2$. The supernatant was collected and stored at B20°C.

Antibody assays: Immunlon 2 (Dynatech laboratories, Chantilly, VA, USA) flat-bottomed plate wells were coated with 0.5 μg of recombinant bacterially expressed HIV-1 p24 polypeptide diluted in PBS and were incubated for 12-15 h at 37EC in a humidity chamber. The plates were then washed 4 times with PBS containing 0.1% Tween 20. The plates were incubated overnight with twofold serially diluted sera in blocking buffer (5% milk powder in PBS) or control anti-p24 antibody at 4EC. After washing 5 times with PBS-Tween 20 (0.1%), the plates were incubated at 37EC with peroxidase conjugated anti-mouse IgG, IgG1, IgG2a or IgG2b antibodies (Sigma) diluted in blocking buffer for 1 h. After washing 5 times with PBS-Tween 20 (0.1%), the amount of bound antibody was determined as follows: phosphate substrate (Sigma) diluted in diethanolamine buffer (Aldrich Chemical, Milwaukee, WI) was applied to the plates incubated for 37EC for 20 min and stop solution (2 N NaOH) was added. OD was taken at 450 nm. The concentration of p24 specific antibodies was calculated from the control monoclonal antibody standard curve and is expressed as arbitrary units.

CTL assays: Splenocytes from mice are stimulated with irradiated (200 Gy) 10ME cells or irradiated (15 Gy) spleen cells osmotically loaded with p24 polypeptide or with the immunodominant peptide AMQMLKETI for 5 days in 30 ml tissue culture media (RPMI with 10% FCS). After washing with media, cells are counted and serially diluted in 96-well microtiter plates. A standard 5 h ^{51}Cr-release assay was performed with 10ME cells as targets at the various effector to target cell ratios. The percent specific lysis is calculated as: [c.p.m. of sample-c.p.m. of spontaneous release)/c.p.m. of maximum release-c.p.m. of spontaneous release)] X 100. Spontaneous release is defined as the mean c.p.m. released from five replicates of $1X10^4$ labeled cells incubated in media alone. Maximum release is defined as the mean c.p.m. released from five replicates of $1X10^4$ labeled cells incubated in media containing Triton X-100.

Another protocol to measure CTL activity is based on detection of IFN-γ-releasing cells by single-cell ELISPOT assay. Briefly, 96-well filtration plates (Millipore, Bedford, MA) were

coated with rat anti-mouse IFN-γ antibody (clone R4-6A2, Pharminogen, San Diego, CA.). Three fold dilution of responder cells in complete PMI-1640 with 10% FCS and 50 units recombinant mouse IL-2 (R&D) per well are added into the wells, along with 10 ME fibroblast 10^5 per well. Cells are incubated for 24-36 hours with or without the peptide AMQMLKETI. After culture, the plates are washed followed by incubation for two hours with biotinylated anti-mouse IFN-γ antibody (clone XMG1.2 Pharminogen). The plates are washed and ExtraAvidin-alkaline Phosphatase (Sigma Cat No E-2636) at dilution 1/2000 in PBS/Tween. After 30 minutes incubation, spots are developed using freshly prepared substrate (BCIP/NBT (Sigma Fast Cat No B-5655) and manually counted to assess CTL activity.

Proliferation assay: Splenocytes from mice obtained 8 weeks after initial immunization are incubated for 3 days at 37EC in 96-well, round-bottom tissue culture plates with an engineered Me10 cell line constitutively expressing HIV-1 p24 in 200 μl of media. [^{3}H] thymidine (0.25μCi; Amersham, Buckingham, UK) incorporation during an 8 h culture is determined using a top count β-scintillation counter (Packard, Downers Grove, IL). The mean stimulation index is calculated as the c.p.m of the splenocytes with antigen divided by c.p.m. of splenocytes alone.

Generation of cell line constitutively presenting HIV-p24 antigen on cell surface: Murine 10ME cells were transfected with the p24-expressing construct pRANT24.

The day before the transfection, 1.5 X 10^5 cells were seeded into 6-well tissue culture plates in 2.5 ml complete media. The cells were 40-60% confluent on the day of transfection. For the transfection, 150 μl of serum free EMEM containing 5 μg of plasmid DNA was mixed well with 75 μg Superfect™ transfection reagent and allowed to stand at RT for 10 min to allow complex formation. Growth medium was aspirated from the cells and the cells washed once with 4 ml PBS. The DNA-Superfect™ mixture was diluted with 1 ml of complete growth medium and added to the cells. They were then re-incubated at 37EC and 5% CO_2 for 3 h after which the medium was aspirated and the cells were washed again with 4 ml PBS. 2.5 ml fresh complete medium was added to the cells and incubation at 37EC and 5% CO_2 was continued for another 48-72 h after which the cell media was harvested. Media was assayed for p24 as described above. Transfected cells

were then selected by adding 0.5 mg/ml of G418 (Gibco/BRL/Life Sciences, Gaithersburg, MD).

6.3 Example 6: Immunizations

In a further experiment, 4 animals per group were injected with naked DNA as follows: Group A received a DNA vector (100ug) but no antigen. Group B reeived a construct encoding p24 (50ug) and a vector (50ug). Group C received a construct encoding p24 (50 ug) and a construct encoding murine MCP-1 (pcmuMCP1, 50ug). Group D received the pTORINO (encodes and expresses a chimera of MCP-1 and p24) (100ug). Mice were injected 3 times at week 0, 2, and 4. mice were bled prior each injection. After the injections, bleeding of the animal was performed every two weeks, for a total of 8 bleedings. To date, results have been obtained for bleed 1 (prior to immunization, i.e. preimmune sera) and at bleed 4 (2 weeks after the last injection).

The following tables show raw OD at 450nm. Methods are as previously described in the application. Sera were diluted 1/10 for this test.

Preimmune sera:

No.	Group A	Group B	Group C	Group D
1	.861	.858	.873	1.102
2	.763	1.316	.952	.706
3	.977	.743	.822	.641
4	1.062	1.377	1.144	.887

Bleed #4:

No.	Group A	Group B	Group C	Group D
1	1.209	.341	1.747	1.453
2	2.402	.826	.911	2.450
3	1.357	1.232	1.431	1.489
4	.936	1.040	1.258	1.988

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various patent and non-patent references are cited herein, the disclosures of which are incorporated by reference in their entireties. Citation of references herein is not an admission that such references are prior art to the present invention.

THE CLAIMS

What is claimed is:

1. A chimeric polypeptide comprising:

   a) one or more chemokine polypeptides selected from the group consisting of:

      i) chemokines; and

      ii) polypeptides within one or more of the following groups: chemokine fragments, chemokine analogues, chemokine derivatives, and chemokine truncation isoforms;

   b) one or more antigenic polypeptides;

   c) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptides.

2. The chimeric polypeptide of claim 1 wherein at least one of the one or more polypeptide linkers connect the one or more antigenic polypeptides to the one or more chemokine polypeptides, in a manner which does not eliminate the antigenicity of the antigenic polypeptides and which does not eliminate the biological activity of the one or more chemokine polypeptides.

3. The chimeric polypeptide of claim 1 wherein at least one of the one or more polypeptide linkers has from 2 to 30 amino acid residues.

4. The chimeric polypeptide of claim 1 wherein at least one of the one or more polypeptide linkers has from 3 to 28 amino acid residues.

5. The chimeric polypeptide of claim 1 wherein at least one of the one or more polypeptide linkers has from 5 to 28 amino acid residues.

6. The chimeric polypeptide of claim 1 wherein at least one of the one or more polypeptide linkers comprises all or a portion of an antibody hinge region.

7. The chimeric polypeptide of claim 1 wherein at least one of the one or more polypeptide linkers comprises a polypeptide hinge region having 50% to 100% sequence homology with a naturally occuring antibody hinge region.

8. The chimeric polypeptide of claim 1 wherein at least one of the one or more polypeptide linkers comprises a polypeptide hinge region having 75% to 100% sequence homology with a naturally occuring antibody hinge region.

9. The chimeric polypeptide of claim 1 wherein at least one of the one or more polypeptide linkers comprises a polypeptide hinge region having 90% to 100% sequence homology with a naturally occuring antibody hinge region.

10. The chimeric polypeptide of claim 6 wherein the antibody hinge region is selected from the group consisting of the hinge regions of the heavy chains of IgG2a and IgG2b.

11. The chimeric polypeptide of claim 1 wherein at least one of the one or more polypeptide linkers is selected from the group consisting of the amino acid sequences of SEQ ID NOS: 19-52.

12. The chimeric polypeptide of claim 1 wherein at least one of the one or more polypeptide linkers comprises the amino acid sequence EPRVPITQNPCPP (SEQ ID NO: 52).

13. The chimeric polypeptide of claim 1 wherein the chimeric polypeptide further comprises a signal peptide which is cleavable from the chimeric polypeptide by enzymatic cleavage.

14. The chimeric polypeptide of claim 1 wherein at least one of the one or more antigenic polypeptides is from a source selected from the group consisting of plants, fungi, protozoa, bacteria, and viruses.

15. The chimeric polypeptide of claim 1 wherein at least one of the one or more antigenic polypeptides is selected from the group consisting of self-antigens, allergens and tumor-associated antigens.

16. The chimeric polypeptide of claim 1 wherein at least one of the one or more antigenic polypeptides is from a bacterium or virus pathogenic to humans.

17. The chimeric polypeptide of claim 1 wherein the one or more antigenic polypeptides include one or more HIV antigens.

18. The chimeric polypeptide of claim 17 wherein the one or more HIV antigens are selected from the group consisting of gag p55, gag p17, gag p5, gag p65, HIV protease, reverse transcriptase, gp120, gp160, gp41, tat, rev, nef, vpu and vif.

19. The chimeric polypeptide of claim 17 wherein the one or more HIV antigens include HIV-1 p24 strain IIIB.

20. The chimeric polypeptide of claim 1 wherein the chemokine polypeptide is selected from a chemokine class selected from the group consisting of: C, CXC, C-C and CX_3C.

21. The chimeric polypeptide of claim 1 wherein the chemokine polypeptide is selected from the group consisting of: Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, Activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EBI1-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, Liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, Thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant;

Monotactin, Activation-induced, Chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, Platelet factor 4, Growth-regulated gene-alpha, Growth-regulated gene-beta, Growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, Granulocyte chemotactic protein 2, lymphotactin, Fractalkine/neurotactin, viral chemokines and functional equivalents thereof.

22. The chimeric polypeptide of claim 1 wherein the chemokine polypeptide is chemotactic for one or more cells selected from the group consisting of dendritic cells, monocytes/ macrophages, B-cells and T cells.

23. The chimeric polypeptide of claim 1 wherein the chemokine polypeptide is selected from the group consisting of MDC, BLC, RANTES, MCP-1 and functional equivalents thereof.

24. The chimeric polypeptide of claim 1 wherein the chemokine polypeptide comprises a human chemokine polypeptide or a functional equivalent thereof.

25. The chimeric polypeptide of claim 1 wherein the chemokine polypeptide comprises a derivative of a chemokine having one or more insertions or substitutions with one or more non-classical amino acids, which derivative has the capacity to enhance an immune response.

26. The chimeric polypeptide of claim 1 wherein the chemokine polypeptide comprises a derivative of a chemokine having at least one conservative substitution in the amino acid sequence, which derivative has the capacity to enhance an immune response.

27. The chimeric polypeptide of claim 1 wherein the chemokine polypeptide comprises a human chemokine.

28. The chimeric polypeptide of claim 1 wherein the chemokine polypeptide comprises an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS: 2, 4, 6 and 8.

29. The chimeric polypeptide of claim 1 wherein the chemokine polypeptide comprises an amino acid sequence selected from the group consisting of:

a) amino acids 25-477 of SEQ ID NO: 2;

b) amino acids 17-493 of SEQ ID NO: 4;

c) amino acids 23-477 of SEQ ID NO: 6; and

d) amino acids 24-528 of SEQ ID NO: 8.

30. A polynucleotide comprising a nucleotide sequence encoding a chimeric polypeptide comprising:

a) one or more chemokine polypeptides selected from the group consisting of:

i) chemokines; and

ii) polypeptides within one or more of the following groups: chemokine fragments, chemokine analogues, chemokine derivatives, and chemokine truncation isoforms;

b) one or more antigenic polypeptides;

c) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptides.

31. The polynucleotide of claim 30 wherein at least one of the one or more polypeptide linkers connects the one or more antigenic polypeptides to the one or more chemokine polypeptides, in a manner which does not eliminate the antigenicity of the antigenic polypeptides and which does not eliminate the biological activity of the one or more chemokine polypeptides.

32. The polynucleotide of claim 30 wherein at least one of the one or more polypeptide linkers has from 2 to 30 amino acid residues.

33. The polynucleotide of claim 30 wherein at least one of the one or more polypeptide linkers has from 3 to 28 amino acid residues.

34. The polynucleotide of claim 30 wherein at least one of the one or more polypeptide linkers has from 5 to 28 amino acid residues.

35. The polynucleotide of claim 30 wherein at least one of the one or more polypeptide linkers comprises all or a portion of an antibody hinge region.

36. The polynucleotide of claim 35 wherein at least one of the one or more polypeptide linkers comprises a polypeptide hinge region having 50% to 100% sequence homology with a naturally occuring antibody hinge region.

37. The polynucleotide of claim 35 wherein at least one of the one or more polypeptide linkers comprises a polypeptide hinge region having 75% to 100% sequence homology with a naturally occuring antibody hinge region.

38. The polynucleotide of claim 35 wherein at least one of the one or more polypeptide linkers comprises a polypeptide hinge region having 90% to 100% sequence homology with a naturally occuring antibody hinge region.

39. The polynucleotide of claim 35 wherein the antibody hinge region is selected from the group consisting of the hinge regions of the heavy chains of IgG2a and IgG2b.

40. The polynucleotide of claim 30 wherein at least one of the one or more polypeptide linkers is selected from the group consisting of the amino acid sequences of SEQ ID NOS: 19-52.

41. The polynucleotide of claim 30 wherein at least one of the one or more polypeptide linkers comprises the amino acid sequence EPRVPITQNPCPP (SEQ ID NO: 52).

42. The polynucleotide of claim 30 wherein the chimeric polypeptide further comprises a signal peptide which is cleavable from the chimeric polypeptide by enzymatic cleavage.

43. The polynucleotide of claim 30 wherein at least one of the one or more antigenic polypeptides is from a source selected from the group consisting of plants, fungi, protozoa, bacteria, and viruses.

44. The polynucleotide of claim 30 wherein at least one of the one or more antigenic polypeptides is selected from the group consisting of self-antigens, allergens and tumor-associated antigens.

45. The polynucleotide of claim 30 wherein at least one of the one or more antigenic polypeptides is from a bacterium or virus pathogenic to humans.

46. The polynucleotide of claim 30 wherein the one or more antigenic polypeptides include one or more HIV antigens.

47. The polynucleotide of claim 46 wherein the one or more HIV antigens are selected from the group consisting of gag p55, gag p17, gag p5, gag p65, HIV protease, reverse transcriptase, gp120, gp160, gp41, tat, rev, nef, vpu and vif.

48. The polynucleotide of claim 46 wherein the one or more HIV antigens include HIV-1 p24 strain IIIB.

49. The polynucleotide of claim 30 wherein the chemokine polypeptide is selected from a chemokine class selected from the group consisting of: C, CXC, C-C and CX_3C.

50. The polynucleotide of claim 30 wherein the chemokine polypeptide is selected from the group consisting of: Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, Activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory

protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EBI1-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, Liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, Thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, Chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, Platelet factor 4, Growth-regulated gene-alpha, Growth-regulated gene-beta, Growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, Granulocyte chemotactic protein 2, lymphotactin, Fractalkine/neurotactin, viral chemokines and functional equivalents thereof.

51. The polynucleotide of claim 30 wherein the chemokine polypeptide is chemotactic for one or more cells selected from the group consisting of dendritic cells, monocytes/ macrophages, B-cells and T cells.

52. The polynucleotide of claim 30 wherein the chemokine polypeptide is selected from the group consisting of MDC, BLC, RANTES, MCP-1 and functional equivalents thereof.

53. The polynucleotide of claim 30 wherein the chemokine polypeptide comprises a human chemokine polypeptide or a functional equivalent thereof.

54. The polynucleotide of claim 30 wherein the chemokine polypeptide comprises a derivative of a chemokine having one or more insertions or substitutions with one or more non-classical amino acids, which derivative has the capacity to enhance an immune response.

55. The polynucleotide of claim 30 wherein the chemokine polypeptide comprises a derivative of a chemokine having at least one conservative substitution in the amino acid sequence, which derivative has the capacity to enhance an immune response.

56. The polynucleotide of claim 30 wherein the chemokine polypeptide comprises a human chemokine.

57. The polynucleotide of claim 30 wherein the chemokine polypeptide comprises an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS: 2, 4, 6 and 8.

58. The polynucleotide of claim 30 wherein the chemokine polypeptide comprises an amino acid sequence selected from the group consisting of:

a) amino acids 25-477 of SEQ ID NO: 2;

b) amino acids 17-493 of SEQ ID NO: 4;

c) amino acids 23-477 of SEQ ID NO: 6; and

d) amino acids 24-528 of SEQ ID NO: 8.

59. The polynucleotide of claim 30 comprising a nucleotide sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS: 1, 3, 5 and 7.

60. An expression vector comprising the polynucleotide of claim 30.

61. A host cell transformed by the expression vector of claim 60, which host cell expresses the chimeric polypeptide.

62. A live vector vaccine comprising the expression vector of claim 61.

63. A method for eliciting an immune response comprising administering to a subject, in an amount effective to enhance an immune response, a chimeric polypeptide and/or a nucleotide encoding a chimeric polypeptide, wherein the chimeric polypeptide comprises:

a) one or more chemokine polypeptides selected from the group consisting of:

i) chemokines; and

ii) polypeptides within one or more of the following groups: chemokine fragments, chemokine analogues, chemokine derivatives, and chemokine truncation isoforms;

b) one or more antigenic polypeptides;

c) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptides.

64. The method of claim 63 wherein the chimeric polypeptide is administered in an immunizingly effective amount.

65. The method of claim 63 wherein the immune response is enhanced relative to an immune response in a corresponding subject to whom a corresponding antigen is administered, alone or attached to a non-chemokine polypeptide.

66. The method of claim 63 wherein at least one of the one or more polypeptide linkers connect the one or more antigenic polypeptides to the one or more chemokine polypeptides, in a manner which does not eliminate the antigenicity of the antigenic polypeptides and which does not eliminate the biological activity of the one or more chemokine polypeptides.

67. The method of claim 63 wherein at least one of the one or more polypeptide linkers has from 2-30 amino acid residues.

68. The method of claim 63 wherein at least one of the one or more polypeptide linkers has from 3-28 amino acid residues.

69. The method of claim 63 wherein at least one of the one or more polypeptide linkers has from 5-28 amino acid residues.

70. The method of claim 63 wherein the chimeric polypeptide further comprises a signal peptide which is cleavable from the chimeric polypeptide by enzymatic cleavage.

71. The method of claim 63 wherein at least one of the one or more antigenic polypeptides is from a source selected from the group consisting of plants, fungi, protozoa, bacteria, and viruses.

72. The method of claim 63 wherein at least one of the one or more antigenic polypeptides is selected from the group consisting of self-antigens, allergens, and tumor-associated antigens.

73. The method of claim 63 wherein at least one of the one or more antigenic polypeptides is from a bacterium or virus pathogenic to humans.

74. The method of claim 63 wherein at least one of the one or more antigenic polypeptides is an HIV antigen.

75. The method of claim 74 wherein the HIV antigen(s) are selected from the group consisting of gag p55, gag p17, gag p5, gag p65, HIV protease, reverse transcriptase, gp120, gp160, gp41, tat, rev, nef, vpu and vif.

76. The method of claim 74 wherein the HIV antigen(s) include HIV-1 p24 strain IIIB.

77. The method of claim 63 wherein the chemokine polypeptide is selected from a chemokine class selected from the group consisting of: C, CXC, C-C and CX_3C.

78. The method of claim 63 wherein the chemokine polypeptide is selected from the group consisting of: Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, Activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its

variants), I-309, EBI1-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, Liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, Thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, Chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, Platelet factor 4, Growth-regulated gene-alpha, Growth-regulated gene-beta, Growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, Granulocyte chemotactic protein 2, lymphotactin, Fractalkine/neurotactin, viral chemokines and functional equivalents thereof.

79. The method of claim 63 wherein the chemokine polypeptide is selected from the group consisting of MDC, BLC, RANTES, and MCP-1 and functional equivalents thereof.

80. The method of claim 63 wherein at least one of the one or more polypeptide linkers comprises an antibody hinge region.

81. The method of claim 63 wherein at least one of the one or more antibody hinge regions are selected from the group consisting of the hinge region of the heavy chain of IgG2a and IgG2b.

82. The chimeric polypeptide of claim 63 wherein at least one of the one or more polypeptide linkers comprises the amino acid sequence EPRVPITQNPCPP (SEQ ID NO: 52).

83. The method of claim 63 wherein at least one of the one or more antibody hinge regions comprises the amino acid sequence is selected from the group consisting of the amino acid sequences of SEQ ID NOS: 19-52.

84. The method of claim 63 wherein the chemokine polypeptide is a derivative of a chemokine having one or more insertions or substitutions with one or more non-classical amino acids, which derivative has the capacity to enhance an immune response.

85. The method of claim 63 wherein the chemokine polypeptide is a derivative of a chemokine having at least one conservative substitution in the amino acid sequence, which derivative has the capacity to enhance an immune response.

86. The method of claim 63 wherein at least one of the one or more chemokine polypeptides is a human chemokine.

87. The method of claim 63 wherein the subject is human.

88. The method of claim 63 wherein the subject is infected or at risk of being infected with HIV virus.

89. The method of claim 63 wherein the chimeric polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6 and 8.

90. The method of claim 63 wherein the chimeric polypeptide comprises an amino acid sequence selected from the group consisting of:

a) amino acids 25-477 of SEQ ID NO: 2;

b) amino acids 17-493 of SEQ ID NO: 4;

c) amino acids 23-477 of SEQ ID NO: 6; and

d) amino acids 24-528 of SEQ ID NO: 8.

91. The method of claim 63 wherein the immune response comprises a humoral response.

92. The method of claim 63 wherein the immune response comprises a cell-mediated response.

93. The method of claim 63 wherein the immune response comprises both a humoral and a cell-mediated response.

94. A pharmaceutical composition comprising a pharmaceutically acceptable carrier in association with the chimeric polypeptide of claim 1 or a nucleotide sequence encoding the chimeric polypeptide of claim 1.

95. The pharmaceutical composition of claim 94 formulated for administration as a vaccine.

96. The pharmaceutical composition of claim 94 wherein the pharmaceutically acceptable carrier is selected from the group consisting of physiological buffer, physiological saline, buffered saline, a slow release carrier, an emulsion and a liposome preparation.

97. The pharmaceutical composition of claim 94 further comprising one or more pharmaceutically acceptable agents selected from the group consisting of: excipients, auxiliary substances, adjuvants, wetting or emulsifying agents, and pH buffering agents.

98. A method of producing one or more chimeric polypeptides comprising:

a) preparing an expression vector comprising a nucleotide sequence encoding a chimeric polypeptide comprising:

i) one or more chemokine polypeptides selected from the group consisting of:

(1) chemokines; and

(2) polypeptides characterized as one or more of the following: fragments of chemokines, analogues of chemokines, derivatives of chemokines, and truncation isoforms of chemokines;

ii) one or more antigenic polypeptides; and

iii) one or more polypeptide linkers connecting the one or more chemokine polypeptides to the one or more antigenic polypeptides;

b) transforming a host cell with the expression vector of (a); and

c) causing the host cell to express the chimeric polypeptide.

99. A polypeptide consisting of an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS: 2, 4, 6 and 8.

100. A polypeptide consisting of an amino acid sequence selected from the group consisting of:

a) amino acids 25-477 of SEQ ID NO: 2;

b) amino acids 17-493 of SEQ ID NO: 4;

c) amino acids 23-477 of SEQ ID NO: 6; and

d) amino acids 24-528 of SEQ ID NO: 8.

101. A polynucleotide encoding an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS: 2, 4, 6 and 8.

102. A polynucleotide comprising a polynucleotide sequence selected from the group consisting of the polynucleotide sequences of SEQ ID NOS: 1, 3, 5 and 7.

103. A polynucleotide encoding an amino acid sequence selected from the group consisting of:

a) amino acids 25-477 of SEQ ID NO: 2;

b) amino acids 17-493 of SEQ ID NO: 4;

c) amino acids 23-477 of SEQ ID NO: 6; and

d) amino acids 24-528 of SEQ ID NO: 8.

104. A chimeric polypeptide having the formula:

C-L-A

wherein:

C is a chemokine polypeptide selected from the group consisting of:

chemokines; and

polypeptides characterized as one or more of the following: chemokine fragments, chemokine analogues, chemokine derivatives, and chemokine truncation isoforms;

A is an antigenic polypeptide;

L is a polypeptide linker which does not eliminate the biological activity of C or the antigenicity of A;

and C, L and A are joined by peptide bonds.

SEQUENCE LISTING

<110> Garzino-Demo, Alfredo

<120> Chimeric Chemokine-Antigen Polypeptides

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gtgataccca tgttttcagc attatcagaa ggagccaccc cacaagattt aaacaccatg 480

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caagcttcac aggaggtaaa aaattggatg acagaaacct tgttggtcca aaatgcgaac 900

ccagattgta agactatttt aaaagcattg ggaccagcgg ctacactaga agaaatgatg 960

acagcatgtc agggagtagg aggacccggc cataaggcaa gagttttggc tgaagcaatg 1020

agccaagtaa caaattcagc taccataatg atgcagagag gcaatttttag gaaccaaaga 1080

aagattgtta agtgtttcaa ttgtggcaaa gaagggcaca cagccagaaa ttgcagggcc 1140

cctaggaaaa agggctgttg gaaatgtgga aaggaaggac accaaatgaa agattgtact 1200

gagagacagg ctaatttttt agggaagatc tggccttcct acaagggaag gccagggaat 1260

tttcttcaga gcagaccaga gccaacagcc ccaccagaag agagcttcag gtctggggta 1320

gagacaacaa ctccccctca gaagcaggag ccgatagaca aggaactgta tcctttaact 1380

tccctcaggt cactctttgg caacgacccc tcgtcacaat aa 1422

<210> 2

<211> 473

<212> PRT

<213> Artificial Sequence

<220>

<221> SIGNAL

<222> (1)..(24)

<223> Signal peptide

<220>

<221> Misc. Feature

<222> (25)..(92)

<223> murine MDC

<220>

<221> Misc. Feature

<222> (93)..(105)

<223> Hinge region of Ig gamma 2a/b as reported for mouse strain C57BL/6

<220>

<221> Misc. Feature

<222> (106)..(473)

<223> p24 core antigen of HIV-1, strain 111B

<220>

<221> Misc. Feature

<222> (25)..(473)

<223> Mature peptide

<220>

<223> Description of Artificial Sequence: Chimeric polypeptide having features as described above

<400> 2

Met Ala Thr Leu Arg Val Pro Leu Leu Val Ala Leu Val Leu Leu Ala
1 5 10 15

Val Ala Ile Gln Thr Ser Asp Ala Gly Pro Tyr Gly Ala Asn Val Glu
20 25 30

Asp Ser Ile Cys Cys Gln Asp Tyr Ile Arg His Pro Leu Pro Ser Arg
35 40 45

Leu Val Lys Glu Phe Phe Trp Thr Ser Lys Ser Cys Arg Lys Pro Gly
50 55 60

Val Val Leu Ile Thr Val Lys Asn Arg Asp Ile Cys Ala Asp Pro Arg
65 70 75 80

Gln Val Trp Val Lys Lys Leu Leu His Lys Leu Ser Glu Pro Arg Val
85 90 95

Pro Ile Thr Gln Asn Pro Cys Pro Pro Pro Ile Val Gln Asn Ile Gln

100 105 110

Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp
115 120 125

Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met
130 135 140

Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met
145 150 155 160

Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu
165 170 175

Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Val His Pro Val His
180 185 190

Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp
195 200 205

Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr
210 215 220

Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile
225 230 235 240

Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu
245 250 255

Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg
260 265 270

Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn
275 280 285

Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys
290 295 300

Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met
305 310 315 320

Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu
325 330 335

Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln
340 345 350

Arg Gly Asn Phe Arg Asn Gln Arg Lys Ile Val Lys Cys Phe Asn Cys
355 360 365

Gly Lys Glu Gly His Thr Ala Arg Asn Cys Arg Ala Pro Arg Lys Lys
370 375 380

Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Thr
385 390 395 400

Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser Tyr Lys Gly
405 410 415

Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro
420 425 430

Glu Glu Ser Phe Arg Ser Gly Val Glu Thr Thr Thr Pro Pro Gln Lys
435 440 445

Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Thr Ser Leu Arg Ser
450 455 460

Leu Phe Gly Asn Asp Pro Ser Ser Gln
465 470

<210> 3
<211> 1488
<212> DNA
<213> Artificial Sequence

<220>
<221> sig_peptide
<222> (1)..(48)
<223> Signal peptide

<220>
<221> misc_feature
<222> (49)..(327)
<223> Murine BLC

<220>
<221> misc_feature
<222> (328)..(364)
<223> Hinge region of Ig gamma 2a/b as reported for mouse strain C57BL/6

<220>

<221> misc_feature

<222> (364)..(1470)

<223> p24 core antigen of HIV-1, strain IIIB

<220>

<221> misc_feature

<222> (1471)..(1473)

<223> Stop codon

<400> 3

atgaggctca gcacagcaac gctgcttctc ctcctggcca gctgcctctc tccaggccac 60

ggtattctgg aagcccatta cacaaactta aaatgtaggt gttctggagt gatttcaact 120

gttgtcggtc taaacatcat agatcggatt caagttacgc cccctgggaa tggctgcccc 180

aaaactgaag ttgtgatctg gaccaagatg aagaaagtta tatgtgtgaa tcctcgtgcc 240

aaatggttac aaagattatt aagacatgtc caaagcaaaa gtctgtcttc aactccccaa 300

gctccagtga gtaagagaag agctgccgag cccagagtgc ccataacaca gaacccctgt 360

cctccaccta tagtgcagaa ccctatagtg cagaacatcc aggggcaaat ggtacatcag 420

gccatatcac ctagaacttt aaatgcatgg gtaaaagtag tagaagagaa ggctttcagc 480

ccagaagtga tacccatgtt ttcagcatta tcagaaggag ccaccccaca agatttaaac 540

accatgctaa acacagtggg gggacatcaa gcagccatgc aaatgttaaa agagaccatc 600

aatgaggaag ctgcagaatg ggatagagtg catccagtgc atgcagggcc tattgcacca 660

ggccagatga gagaaccaag gggaagtgac atagcaggaa ctactagtac ccttcaggaa 720

caaataggat ggatgacaaa taatccacct atcccagtag gagaaattta taaaagatgg 780

ataatcctgg gattaaataa aatagtaaga atgtatagcc ctaccagcat tctggacata 840

agacaaggac caaaggaacc ctttagagac tatgtagacc ggttctataa aactctaaga 900

gccgagcaag cttcacagga ggtaaaaaat tggatgacag aaaccttgtt ggtccaaaat 960

gcgaacccag attgtaagac tattttaaaa gcattgggac cagcggctac actagaagaa 1020

atgatgacag catgtcaggg agtaggagga cccggccata aggcaagagt tttggctgaa 1080

gcaatgagcc aagtaacaaa ttcagctacc ataatgatgc agagaggcaa ttttaggaac 1140

caaaagaaaa ttgttaagtg tttcaattgt ggcaaagaag ggcacacagc cagaaattgc 1200

agggcccctа ggaaaaaggg ctgttggaaa tgtggaaagg aaggacacca aatgaaagat 1260

tgtactgaga gacaggctaa ttttttaggg aagatctggc cttcctacaa gggaaggcca 1320

gggaattttc ttcagagcag accagagcca acagccccac cagaagagag cttcaggtct 1380

ggggtagaga caacaactcc ccctcagaag caggagccga tagacaagga actgtatcct 1440

ttaacttccc tcaggtcact ctttggcaac gaccccтcgt cacaataa 1488

<210> 4

<211> 490

<212> PRT

<213> Artificial Sequence

<220>

<221> Misc. Feature

<222> (1)..(16)

<223> Signal peptide

<220>

<221> Misc. Feature

<222> (17)..(109)

<223> murine BLC

<220>

<221> Misc. Feature

<222> (110)..(121)

<223> Hinge region of Ig gamma 2a/b as reported for mouse strain C57BL/6

as reported for mouse strain C57BL/6

<220>

<221> Misc. Feature

<222> (122)..(490)

<223> p24 core antigen of HIV-1, strain IIIB

<220>

<221> Misc. Feature

<222> (17)..(490)

<223> Mature peptide

<220>

<223> Description of Artificial Sequence: chimeric polypeptide having features as described above

<400> 4

Met Arg Leu Ser Thr Ala Thr Leu Leu Leu Leu Leu Ala Ser Cys Leu
1 5 10 15

Ser Pro Gly His Gly Ile Leu Glu Ala His Tyr Thr Asn Leu Lys Cys
20 25 30

Arg Cys Ser Gly Val Ile Ser Thr Val Val Gly Leu Asn Ile Ile Asp
35 40 45

Arg Ile Gln Val Thr Pro Pro Gly Asn Gly Cys Pro Lys Thr Glu Val
50 55 60

Val Ile Trp Thr Lys Met Lys Lys Val Ile Cys Val Asn Pro Arg Ala
65 70 75 80

Lys Trp Leu Gln Arg Leu Leu Arg His Val Gln Ser Lys Ser Leu Ser
85 90 95

Ser Thr Pro Gln Ala Pro Val Ser Lys Arg Arg Ala Ala Glu Pro Arg
100 105 110

Val Pro Ile Thr Gln Asn Pro Cys Pro Pro Pro Ile Val Gln Asn Ile
115 120 125

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Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala
    130                 135                 140

Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro
145                 150                 155                 160

Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr
                165                 170                 175

Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys
            180                 185                 190

Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Val His Pro Val
        195                 200                 205

His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser
    210                 215                 220

Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met
225                 230                 235                 240

Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile
                245                 250                 255

Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile
            260                 265                 270

Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp
        275                 280                 285
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Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys
290 295 300

Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys
305 310 315 320

Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met
325 330 335

Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val
340 345 350

Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met
355 360 365

Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Ile Val Lys Cys Phe Asn
370 375 380

Cys Gly Lys Glu Gly His Thr Ala Arg Asn Cys Arg Ala Pro Arg Lys
385 390 395 400

Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys
405 410 415

Thr Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser Tyr Lys
420 425 430

Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro
435 440 445

Pro Glu Glu Ser Phe Arg Ser Gly Val Glu Thr Thr Thr Pro Pro Gln

450 455 460

Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Thr Ser Leu Arg
465 470 475 480

Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln
485 490

<210> 5
<211> 1434
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<222> (1)..(69)
<223> Signal peptide

<220>
<221> misc_feature
<222> (70)..(273)
<223> mature murine RANTES

<220>
<221> misc_feature
<222> (274)..(312)
<223> Hinge region of Ig gamma 2a/b as reported for mouse strain C57BL/6

<220>
<221> misc_feature

<222> (313)..(1416)

<223> p24 core antigen of HIV-1, strain IIIB

<220>

<221> misc_feature

<222> (1417)..(1419)

<223> Stop codon

<400> 5

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gcacctgcct caccatatgg ctcggacacc actccctgct gctttgccta cctctcccta 120

gagctgcctc gtgcccacgt caaggagtat ttctacacca gcagcaagtg ctccaatctt 180

gcagtcgtgt ttgtcactcg aaggaaccgc caagtgtgtg ccaacccaga gaagaagtgg 240

gttcaagaat acatcaacta tttggagatg agcgagccca gagtgcccat aacacagaac 300

ccctgtcctc cacctatagt gcagaaccct atagtgcaga acatccaggg gcaaatggta 360

catcaggcca tatcacctag aactttaaat gcatgggtaa aagtagtaga agagaaggct 420

ttcagcccag aagtgatacc catgttttca gcattatcag aaggagccac cccacaagat 480

ttaaacacca tgctaaacac agtgggggga catcaagcag ccatgcaaat gttaaaagag 540

accatcaatg aggaagctgc agaatgggat agagtgcatc cagtgcatgc agggcctatt 600

gcaccaggcc agatgagaga accaagggga agtgacatag caggaactac tagtaccctt 660

caggaacaaa taggatggat gacaaataat ccacctatcc cagtaggaga aatttataaa 720

agatggataa tcctgggatt aaataaaata gtaagaatgt atagccctac cagcattctg 780

gacataagac aaggaccaaa ggaacccttt agagactatg tagaccggtt ctataaaact 840

ctaagagccg agcaagcttc acaggaggta aaaaattgga tgacagaaac cttgttggtc 900

caaaatgcga acccagattg taagactatt ttaaaagcat tgggaccagc ggctacacta 960

gaagaaatga tgacagcatg tcagggagta ggaggacccg gccataaggc aagagttttg 1020

gctgaagcaa tgagccaagt aacaaattca gctaccataa tgatgcagag aggcaatttt 1080

aggaaccaaa gaaagattgt taagtgtttc aattgtggca aagaagggca cacagccaga 1140

aattgcaggg cccctaggaa aaagggctgt tggaaatgtg gaaaggaagg acaccaaatg 1200

aaagattgta ctgagagaca ggctaatttt ttagggaaga tctggccttc ctacaaggga 1260

aggccaggga attttcttca gagcagacca gagccaacag ccccaccaga agagagcttc 1320

aggtctgggg tagagacaac aactcccccc cagaagcagg agccgataga caaggaactg 1380

tatcctttaa cttccctcag gtcactcttt ggcaacgacc cctcgtcaca ataa 1434

<210> 6

<211> 472

<212> PRT

<213> Artificial Sequence

<220>

<221> SIGNAL

<222> (1)..(23)

<223> signal

<220>

<221> Misc. Feature

<222> (24)..(91)

<223> murine RANTES

<220>

<221> Misc. Feature

<222> (92)..(104)

<223> Hinge region of Ig gamma 2a/b as reported for mouse strain C57BC/6

<220>

<221> Misc. Feature

<222> (105)..(472)

<223> p24 core antigen of HIV-1, strain IIIB

<220>

<221> Misc. Feature

<222> (23)..(472)

<223> Mature peptide

<220>

<223> Description of Artificial Sequence: chimeric polypeptide having features as described above

<400> 6

Met Lys Ile Ser Ala Ala Ala Leu Thr Ile Ile Leu Thr Ala Ala Ala
1 5 10 15

Leu Cys Thr Pro Ala Pro Ala Ser Pro Tyr Gly Ser Asp Thr Thr Pro
20 25 30

Cys Cys Phe Ala Tyr Leu Ser Leu Glu Leu Pro Arg Ala His Val Lys
35 40 45

Glu Tyr Phe Tyr Thr Ser Ser Lys Cys Ser Asn Leu Ala Val Val Phe
50 55 60

Val Thr Arg Arg Asn Arg Gln Val Cys Ala Asn Pro Glu Lys Lys Trp
65 70 75 80

Val Gln Glu Tyr Ile Asn Tyr Leu Glu Met Ser Glu Pro Arg Val Pro
85 90 95

Ile Thr Gln Asn Pro Cys Pro Pro Pro Ile Val Gln Asn Ile Gln Gly
100 105 110

Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val
115 120 125

Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe

130 135 140

Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu
145 150 155 160

Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr
165 170 175

Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Val His Pro Val His Ala
180 185 190

Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile
195 200 205

Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn
210 215 220

Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu
225 230 235 240

Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp
245 250 255

Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe
260 265 270

Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp
275 280 285

Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr
290 295 300

Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr
305 310 315 320

Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala
325 330 335

Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln Arg
340 345 350

Gly Asn Phe Arg Asn Gln Arg Lys Ile Val Lys Cys Phe Asn Cys Gly
355 360 365

Lys Glu Gly His Thr Ala Arg Asn Cys Arg Ala Pro Arg Lys Lys Gly
370 375 380

Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Thr Glu
385 390 395 400

Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser Tyr Lys Gly Arg
405 410 415

Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu
420 425 430

Glu Ser Phe Arg Ser Gly Val Glu Thr Thr Thr Pro Pro Gln Lys Gln
435 440 445

Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Thr Ser Leu Arg Ser Leu
450 455 460

Phe Gly Asn Asp Pro Ser Ser Gln
465 470

<210> 7
<211> 1590
<212> DNA
<213> Artificial Sequence

<220>
<221> sig_peptide
<222> (1)..(69)
<223> Signal peptide

<220>
<221> misc_feature
<222> (70)..(444)
<223> Murine MCP-1/JE

<220>
<221> misc_feature
<222> (445)..(483)
<223> Hinge region of Ig gamma 2a/b as reported for mouse strain C57BL/6

<220>
<221> misc_feature
<222> (484)..(1587)
<223> p24 core antigen of HIV-1, strain IIIB

<220>
<221> misc_feature
<222> (1588)..(1590)
<223> Stop codon

<400> 7

atgcaggtcc ctgtcatgct tctgggcctg ctgttcacag ttgccggctg gagcatccac 60

gtgttggctc agccagatgc agttaacgcc ccactcacct gctgctactc attcaccagc 120

aagatgatcc caatgagtag gctggagagc tacaagagga tcaccagcag caggtgtccc 180

aaagaagctg tagtttttgt caccaagctc aagagagagg tctgtgctga ccccaagaag 240

gaatgggtcc agacatacat taaaaacctg gatcggaacc aaatgagatc agaacctaca 300

actttattta aaactgcatc tgccctaagg tcttcagcac ctttgaatgt gaagttgacc 360

cgtaaatctg aagctaatgc atccactacc ttttccacaa ccacctcaag cacttctgta 420

ggagtgacca gtgtgacagt gaacgagccc agagtgccca taacacagaa cccctgtcct 480

ccacctatag tgcagaacat ccaggggcaa atggtacatc aggccatatc acctagaact 540

ttaaatgcat gggtaaaagt agtagaagag aaggctttca gcccagaagt gatacccatg 600

ttttcagcat tatcagaagg agccacccca caagatttaa acaccatgct aaacacagtg 660

gggggacatc aagcagccat gcaaatgtta aaagagacca tcaatgagga agctgcagaa 720

tgggatagag tgcatccagt gcatgcaggg cctattgcac caggccagat gagagaacca 780

aggggaagtg acatagcagg aactactagt acccttcagg aacaaatagg atggatgaca 840

aataatccac ctatcccagt aggagaaatt tataaaagat ggataatcct gggattaaat 900

aaaatagtaa gaatgtatag ccctaccagc attctggaca taagacaagg accaaaggaa 960

ccctttagag actatgtaga ccggttctat aaaactctaa gagccgagca agcttcacag 1020

gaggtaaaaa attggatgac agaaaccttg ttggtccaaa atgcgaaccc agattgtaag 1080

actattttaa aagcattggg accagcggct acactagaag aaatgatgac agcatgtcag 1140

ggagtaggag gacccggcca taaggcaaga gttttggctg aagcaatgag ccaagtaaca 1200

aattcagcta ccataatgat gcagagaggc aattttagga accaaagaaa gattgttaag 1260

tgtttcaatt gtggcaaaga agggcacaca gccagaaatt gcagggcccc taggaaaaag 1320

ggctgttgga aatgtggaaa ggaaggacac caaatgaaag attgtactga gagacaggct 1380

aatttttag ggaagatctg gccttcctac aagggaaggc cagggaattt tcttcagagc 1440

agaccagagc caacagcccc accagaagag agcttcaggt ctggggtaga gacaacaact 1500

ccccctcaga agcaggagcc gatagacaag gaactgtatc ctttaacttc cctcaggtca 1560

ctctttggca acgacccctc gtcacaataa 1590

<210> 8

<211> 528

<212> PRT

<213> Artificial Sequence

<220>

<221> SIGNAL

<222> (1)..(23)

<223> signal

<220>

<221> Misc. Feature

<222> (24)..(148)

<223> murine MCP-1/JE

<220>

<221> Misc. Feature

<222> (149)..(161)

<223> Hinge region of Ig gamma 2a/b as reported for mouse strain C57BL/6

<220>

<221> Misc. Feature

<222> (162)..(528)

<223> p24 core antigen of HIV-1, strain IIIB

<220>

<221> Misc. Feature

<222> (23)..(528)

<223> Mature peptide

<220>

<223> Description of Artificial Sequence: chimeric polypeptide having features as described above

<400> 8

Met Gln Val Pro Val Met Leu Leu Gly Leu Leu Phe Thr Val Ala Gly
1 5 10 15

Trp Ser Ile His Val Leu Ala Gln Pro Asp Ala Val Asn Ala Pro Leu
20 25 30

Thr Cys Cys Tyr Ser Phe Thr Ser Lys Met Ile Pro Met Ser Arg Leu
35 40 45

Glu Ser Tyr Lys Arg Ile Thr Ser Ser Arg Cys Pro Lys Glu Ala Val
50 55 60

Val Phe Val Thr Lys Leu Lys Arg Glu Val Cys Ala Asp Pro Lys Lys
65 70 75 80

Glu Trp Val Gln Thr Tyr Ile Lys Asn Leu Asp Arg Asn Gln Met Arg
85 90 95

Ser Glu Pro Thr Thr Leu Phe Lys Thr Ala Ser Ala Leu Arg Ser Ser
100 105 110

Ala Pro Leu Asn Val Lys Leu Thr Arg Lys Ser Glu Ala Asn Ala Ser
115 120 125

Thr Thr Phe Ser Thr Thr Thr Ser Ser Thr Ser Val Gly Val Thr Ser
130 135 140

Val Thr Val Asn Glu Pro Arg Val Pro Ile Thr Gln Asn Pro Cys Pro
145 150 155 160

Pro Pro Ile Val Gln Asn Ile Gln Gly Gln Met Val His Gln Ala Ile
165 170 175

Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala
180 185 190

Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala
195 200 205

Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln
210 215 220

Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu
225 230 235 240

Trp Asp Arg Val His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln
245 250 255

Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu
260 265 270

Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly
275 280 285

Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg
290 295 300

Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu
305 310 315 320

Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu
325 330 335

Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val
340 345 350

Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro
355 360 365

Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly
370 375 380

Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr
385 390 395 400

Asn Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg
405 410 415

Lys Ile Val Lys Cys Phe Asn Cys Gly Lys Glu Gly His Thr Ala Arg
420 425 430

Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu
435 440 445

Gly His Gln Met Lys Asp Cys Thr Glu Arg Gln Ala Asn Phe Leu Gly
450 455 460

Lys Ile Trp Pro Ser Tyr Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser
465 470 475 480

Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Ser Gly Val
485 490 495

Glu Thr Thr Thr Pro Pro Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu
500 505 510

Tyr Pro Leu Thr Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser
515 520 525

Gln

<210> 9
<211> 20
<212> PRT
<213> Murinae gen. sp.

<220>
<223> Description of Artificial Sequence: Describe artificial Sequence here

<400> 9
Met Lys Ile Ser Ala Ala Ala Leu Thr Ile Ile Leu Thr Ala Ala Ala
1 5 10 15

Leu Cys Thr Pro Ala Pro Ala
20

<210> 10
<211> 16
<212> PRT
<213> Murinae gen. sp.

<400> 10
Met Arg Leu Ser Thr Ala Thr Leu Leu Leu Leu Leu Ala Ser Cys Leu

1 5 10 15

<210> 11

<211> 24

<212> PRT

<213> Murinae gen. sp.

<400> 11

Met Ala Thr Leu Arg Val Pro Leu Leu Val Ala Leu Val Leu Leu Ala

1 5 10 15

Val Ala Ile Gln Thr Ser Asp Ala

20

<210> 12

<211> 23

<212> PRT

<213> Murinae gen. sp.

<400> 12

Met Gln Val Pro Val Met Leu Leu Gly Leu Leu Phe Thr Val Ala Gly

1 5 10 15

Trp Ser Ile His Val Leu Ala

20

<210> 13

<211> 368

<212> PRT

<213> Human immunodeficiency virus type 1

<400> 13

Pro Ile Val Gln Asn Ile Gln Gly Gln Met Val His Gln Ala Ile Ser
1 5 10 15

Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe
20 25 30

Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr
35 40 45

Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala
50 55 60

Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp
65 70 75 80

Asp Arg Val His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met
85 90 95

Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln
100 105 110

Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu
115 120 125

Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met
130 135 140

Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro
145 150 155 160

Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln
165 170 175

Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln
180 185 190

Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala
195 200 205

Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro
210 215 220

Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn
225 230 235 240

Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys
245 250 255

Ile Val Lys Cys Phe Asn Cys Gly Lys Glu Gly His Thr Ala Arg Asn
260 265 270

Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly
275 280 285

His Gln Met Lys Asp Cys Thr Glu Arg Gln Ala Asn Phe Leu Gly Lys
290 295 300

Ile Trp Pro Ser Tyr Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg

305 310 315 320

Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Ser Gly Val Glu
325 330 335

Thr Thr Thr Pro Pro Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr
340 345 350

Pro Leu Thr Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln
355 360 365

<210> 14

<211> 69

<212> PRT

<213> Murinae gen. sp.

<400> 14

Ala Ser Pro Tyr Gly Ser Asp Thr Thr Pro Cys Cys Phe Ala Tyr Leu
1 5 10 15

Ser Leu Glu Leu Pro Arg Ala His Val Lys Glu Tyr Phe Tyr Thr Ser
20 25 30

Ser Lys Cys Ser Asn Leu Ala Val Val Phe Val Thr Arg Arg Asn Arg
35 40 45

Gln Val Cys Ala Asn Pro Glu Lys Lys Trp Val Gln Glu Tyr Ile Asn

50 55 60

Tyr Leu Glu Met Ser
65

<210> 15
<211> 93
<212> PRT
<213> Murinae gen. sp.

<400> 15
Ser Pro Gly His Gly Ile Leu Glu Ala His Tyr Thr Asn Leu Lys Cys
1 5 10 15

Arg Cys Ser Gly Val Ile Ser Thr Val Val Gly Leu Asn Ile Ile Asp
20 25 30

Arg Ile Gln Val Thr Pro Pro Gly Asn Gly Cys Pro Lys Thr Glu Val
35 40 45

Val Ile Trp Thr Lys Met Lys Lys Val Ile Cys Val Asn Pro Arg Ala
50 55 60

Lys Trp Leu Gln Arg Leu Leu Arg His Val Gln Ser Lys Ser Leu Ser
65 70 75 80

Ser Thr Pro Gln Ala Pro Val Ser Lys Arg Arg Ala Ala
85 90

<210> 16

<211> 68

<212> PRT

<213> Murinae gen. sp.

<400> 16

Gly Pro Tyr Gly Ala Asn Val Glu Asp Ser Ile Cys Cys Gln Asp Tyr
1 5 10 15

Ile Arg His Pro Leu Pro Ser Arg Leu Val Lys Glu Phe Phe Trp Thr
20 25 30

Ser Lys Ser Cys Arg Lys Pro Gly Val Val Leu Ile Thr Val Lys Asn
35 40 45

Arg Asp Ile Cys Ala Asp Pro Arg Gln Val Trp Val Lys Lys Leu Leu
50 55 60

His Lys Leu Ser
65

<210> 17

<211> 125

<212> PRT

<213> Murinae gen. sp.

<400> 17

Gln Pro Asp Ala Val Asn Ala Pro Leu Thr Cys Cys Tyr Ser Phe Thr
1 5 10 15

Ser Lys Met Ile Pro Met Ser Arg Leu Glu Ser Tyr Lys Arg Ile Thr
20 25 30

Ser Ser Arg Cys Pro Lys Glu Ala Val Val Phe Val Thr Lys Leu Lys
35 40 45

Arg Glu Val Cys Ala Asp Pro Lys Lys Glu Trp Val Gln Thr Tyr Ile
50 55 60

Lys Asn Leu Asp Arg Asn Gln Met Arg Ser Glu Pro Thr Thr Leu Phe
65 70 75 80

Lys Thr Ala Ser Ala Leu Arg Ser Ser Ala Pro Leu Asn Val Lys Leu
85 90 95

Thr Arg Lys Ser Glu Ala Asn Ala Ser Thr Thr Phe Ser Thr Thr Thr
100 105 110

Ser Ser Thr Ser Val Gly Val Thr Ser Val Thr Val Asn
115 120 125

<210> 18

<211> 125

<212> PRT

<213> Murinae gen. sp.

<400> 18

Gln Pro Asp Ala Val Asn Ala Pro Leu Thr Cys Cys Tyr Ser Phe Thr
1 5 10 15

Ser Lys Met Ile Pro Met Ser Arg Leu Glu Ser Tyr Lys Arg Ile Thr
20 25 30

Ser Ser Arg Cys Pro Lys Glu Ala Val Val Phe Val Thr Lys Leu Lys
35 40 45

Arg Glu Val Cys Ala Asp Pro Lys Lys Glu Trp Val Gln Thr Tyr Ile
50 55 60

Lys Asn Leu Asp Arg Asn Gln Met Arg Ser Glu Pro Thr Thr Leu Phe
65 70 75 80

Lys Thr Ala Ser Ala Leu Arg Ser Ser Ala Pro Leu Asn Val Lys Leu
85 90 95

Thr Arg Lys Ser Glu Ala Asn Ala Ser Thr Thr Phe Ser Thr Thr Thr
100 105 110

Ser Ser Thr Ser Val Gly Val Thr Ser Val Thr Val Asn
115 120 125

<210> 19

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Polypeptide linker

<400> 19

Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser
1 5 10

<210> 20

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Polypeptide linker

<400> 20

Ala Gly Ser Ala Gly Ser Ala Gly Ser Ala Gly Ser
1 5 10

<210> 21

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Polypeptide linker

<400> 21

Gly Gly Ser Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 22

<211> 3

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Polypeptide linker

<400> 22

Pro Gly Ser
1

<210> 23

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Polypeptide linker

<400> 23

Ile Ala Lys Ala Phe Lys Asn
1 5

<210> 24

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Polypeptide linker

<400> 24

Lys Glu Ser Gly Ser Val Ser Ser Glu Gln Leu Ala Gln Phe Arg Ser
1 5 10 15

Leu Asp

<210> 25

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Polypeptide linker

<400> 25

Val Arg Gly Ser Pro Ala Ile Asn Val Ala Val His Val Phe
1 5 10

<210> 26

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Polypeptide linker constructed primarily of a helical segment of human hemoglobin

<400> 26

Ala Gln Gly Thr Leu Ser Pro Ala Asp Lys Thr Asn Val Lys Ala Ala
1 5 10 15

Trp Gly Lys Val Met Thr
20

<210> 27

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Polypeptide linker

<400> 27

Val Glu Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly
1 5 10 15

Val Asp

<210> 28

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Polypeptide linker

<400> 28

Gly Ser Ala Ser Ala Pro Lys Leu Glu Glu Gly Glu Phe Ser Glu Ala
1 5 10 15

Arg Glu

<210> 29

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Polypeptide linker

<400> 29

Gly Ser Thr Ser Gly Ser Gly Xaa Pro Gly Ser Gly Glu Gly Ser Thr

1 5 10 15

Lys Gly

<210> 30

<211> 10

<212> PRT

<213> Homo sapiens

<400> 30

Glu Pro Lys Ser Cys Asp Lys Thr His Thr

1 5 10

<210> 31

<211> 5

<212> PRT

<213> Homo sapiens

<400> 31

Cys Pro Pro Cys Pro

1 5

<210> 32

<211> 8

<212> PRT

<213> Homo sapiens

<400> 32

Ala Pro Glu Leu Leu Gly Gly Pro

1 5

<210> 33

<211> 3

<212> PRT

<213> Homo sapiens

<400> 33

Glu Arg Lys

1

<210> 34

<211> 9

<212> PRT

<213> Homo sapiens

<400> 34

Cys Cys Val Glu Cys Pro Pro Cys Pro

1 5

<210> 35

<211> 7

<212> PRT

<213> Homo sapiens

<400> 35

Ala Pro Pro Val Ala Gly Pro
1 5

<210> 36
<211> 12
<212> PRT
<213> Homo sapiens

<400> 36
Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr
1 5 10

<210> 37
<211> 5
<212> PRT
<213> Homo sapiens

<400> 37
Cys Pro Arg Cys Pro
1 5

<210> 38
<211> 15
<212> PRT
<213> Homo sapiens

<400> 38
Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro

1 5 10 15

<210> 39

<211> 8

<212> PRT

<213> Homo sapiens

<400> 39

Ala Pro Glu Leu Leu Gly Gly Pro

1 5

<210> 40

<211> 4

<212> PRT

<213> Homo sapiens

<400> 40

Glu Pro Lys Ser

1

<210> 41

<211> 11

<212> PRT

<213> Homo sapiens

<400> 41

Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro

1 5 10

<210> 42

<211> 8

<212> PRT

<213> Homo sapiens

<400> 42

Ala Pro Glu Leu Leu Gly Gly Pro

1 5

<210> 43

<211> 7

<212> PRT

<213> Homo sapiens

<400> 43

Glu Ser Lys Tyr Gly Pro Pro

1 5

<210> 44

<211> 5

<212> PRT

<213> Homo sapiens

<400> 44

Cys Pro Ser Cys Pro

1 5

<210> 45

<211> 8

<212> PRT

<213> Homo sapiens

<400> 45

Ala Pro Glu Phe Leu Gly Gly Pro

1 5

<210> 46

<211> 6

<212> PRT

<213> Homo sapiens

<400> 46

Val Pro Arg Asp Cys Gly

1 5

<210> 47

<211> 7

<212> PRT

<213> Homo sapiens

<400> 47

Cys Lys Pro Cys Ile Cys Thr

1 5

<210> 48

<211> 6

<212> PRT

<213> Homo sapiens

<400> 48

Val Pro Ser Glu Val Ser

1 5

<210> 49

<211> 9

<212> PRT

<213> Homo sapiens

<400> 49

Glu Pro Arg Gly Pro Thr Ile Lys Pro

1 5

<210> 50

<211> 7

<212> PRT

<213> Homo sapiens

<400> 50

Cys Pro Pro Cys Lys Cys Pro

1 5

<210> 51

<211> 8

<212> PRT

<213> Homo sapiens

<400> 51

Ala Pro Asn Leu Leu Gly Gly Pro

1 5

<210> 52

<211> 13

<212> PRT

<213> Murinae gen. sp.

<400> 52

Glu Pro Arg Val Pro Ile Thr Gln Asn Pro Cys Pro Pro

1 5 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/16598

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :530/350, 388.1; 536/23.5; 435/320.1, 325; 514/2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 388.1; 536/23.5; 435/320.1, 325; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST CAPLUS BIOSIS EMBASE REGISTRY

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHALLITA-EID et al. A RANTES-antibody fusion protein retains antigen specificity and chemokine function. Journal of Immunology. 01 October 1998, Vol. 161, Number 7, pages 3729-3736, see entire document.	1-104
Y	US 5,650,150 A (GILLIES) 22 July 1997 (22.07.97), see entire document.	1-104
Y	US 5,824,782 A (HOLZER et al) 20 October 1998 (20.10.98), see entire document.	1-104

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 OCTOBER 2000	Date of mailing of the international search report 17 NOV 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JOSEPH MURPHY Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/16598

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 12, 28-29,58-62,82-83,90,100-104 (in-part)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/16598

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

A61K 38/00; C07H 21/04; C07K 14/00, 16/00; C12N 5/00, 5/06, 15/63

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE
2. Where no meaningful search could be carried out, specifically:

Claims 12, 28-29,58-62, 82-83, 90, 100-104 are unsearchable to the extent that they require reference to the specified sequences from the sequence listing. Because Applicant failed to furnish a machine-readable copy of the sequence listing that contained no errors, no meaningful search of the sequences per se can be carried out by this Authority. However, the subject matter of the claims has been searched to the extent possible with reference to the balance of the description.